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FROM MOVEMENT TO SKILL – NEURAL AND BEHAVIORAL MECHANISMS OF MOTOR SEQUENCE LEARNING

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Front cover:

An illustration of the beauty and complexity of motor skills.

The photograph is called *Back Dive*. Photographer: Harold Edgerton, 1954

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From Movement to Skill – Neural and Behavioral Mechanisms of Motor Sequence Learning

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To Peter De Weerd

ABSTRACT

How do we learn new movements? The simple answer to this question is *Through practice!* Yet, a better response might be *What aspect of motor learning are we talking about?* Our capacity for learning new skills and for combining movements into new sequences is virtually unlimited. In contrast, our understanding of the mechanisms behind motor skill learning is still rather sparse. One part of the problem is that the topic of motor skill learning can be approached from many different angles. An athlete might ask *How should I practice?* or *Which strategies work best?*, the neuroscientist might wonder *How does the brain form new memories for a movement and where are they stored?*, while the child might ask *Why does it take grandpa so long to swipe the unlock pattern on his smartphone?* This thesis will explore some of the behavioral and neural mechanisms of motor skill learning.

One form of motor skill learning is sequence learning, i.e., learning to produce the right movements at the right time and order. Motor sequences have been studied extensively, since response times for individual movement elements (usually key-presses) can be precisely measured and different sequence properties, such as complexity and familiarity, can be easily manipulated. In this thesis, I made use of different sequence learning paradigms to explore 1) how different practice formats affect how flexibly we can use the acquired knowledge in related tasks (**Study I**), 2) how the information for different motor sequences is represented in the brain (**Study II**), and 3) how a specific brain area, the pre-supplementary motor area (preSMA), is involved in chunking, i.e., grouping individual key-presses into movement units (**Study III**).

In **Study I** we assigned participants to two different groups that practiced an implicit sequence learning task either via constant practice (i.e., constantly repeating the same sequence) or via variable practice (i.e., alternating between two different sequences). We found that variable practice led to better performance on a subsequent transfer test, where participants had to perform an entirely different sequence.

In **Study II** we found that familiar (trained) and novel motor sequences are represented by different patterns of neural activity, even in brain areas that did not change their mean level of activity for either sequence type. Moreover, we observed that the patterns of neural activity were related to the patterns of behavioral performance; sequences that were performed at similar speeds also evoked similar patterns of brain activity.

In **Study III** we demonstrated that transcranial magnetic stimulation of the preSMA increased response times for the next sequence element, but only under the demanding condition where the next response required both the initiation of a new chunk and a switch between hands.

Together, these studies show how different practice strategies affect skill generalizability and how task difficulty and proficiency shape the neural implementation of motor skills.

LIST OF SCIENTIFIC PAPERS

I. **Müssgens, D.M.** & Ullén, F. (2015). Transfer in Motor Sequence Learning: Effects of Practice Schedule and Sequence Context. *Frontiers in Human Neuroscience*, *9*(642), 1-13.

II. **Muessgens, D.M.**, Baker, C.I., & Ullén, F. Cortical Representations of Trained and Novel Motor Sequences. *Manuscript.*

III. **Muessgens, D.**, Thirugnanasambandam, N., Shitara, H., Popa, T., & Hallett, M. (2016). *Journal of Neurophysiology, 116*, 2637-2646.

CONTENTS

LIST OF ABBREVIATIONS

AC – PC plane	Plane between anterior and posterior commissure
ANOVA	Analysis of Variance
BOLD	Blood oxygenation level dependent
BS	Between-subjects
CI	Contextual Interference
cTBS	Continuous theta burst stimulation
dPMC	Dorsal premotor cortex
DSP	Discrete sequence production
dTMS	Double-pulse transcranial magnetic stimulation
EMG	electromyography
FDI	First dorsal interosseous
fMRI	Functional magnetic resonance imaging
FWE	Family-wise error
FWHM	Full width half maximum
GLM	General linear model
ISI	Inter-stimulus interval
M1	Primary motor cortex
MEP	Motor evoked potential
MRI	Magnetic resonance imaging
PMC	Premotor cortex
preSMA	Pre-supplementary motor area
rmANOVA	Repeated measures ANOVA
RMT	Resting motor threshold
ROI	Region of interest
RSA	Representational similarity analysis
RSI	Response-to-stimulus interval
RT	Response time
SD	Standard deviation
SE	Standard error
SMA	Supplementary motor area
SRT	Serial response time
TMS	Transcranial magnetic stimulation
vPMC	Ventral premotor cortex
WS	Within-subjects

1 INTRODUCTION

Practice makes perfect. This phrase captures our everyday life experience with motor skill learning so neatly, that the question of why practice is necessary seems almost trivial. You just know that if you want to learn to play a musical instrument or to dance Salsa, you will have to practice for many hours. Similarly, if you want to learn to do a back dive, it will not help you to study the cover of this thesis (nor will reading the following pages help you, sorry!), you will have to actually get your feet wet.

But what exactly happens when we learn a new skill? Obviously, the question of what is being learned depends a lot on the type of skill that we are practicing. For a back dive, we would have to learn how to activate our muscles in a coordinated way to gain the necessary height and momentum to rotate our entire body. Thus, we would have to acquire a new set of movement kinematics. On the other hand, for learning how to type quickly on a keyboard, we already know how to perform the individual elements (pressing a key), but we need to learn which finger corresponds to which key and in which order to press them. The latter skill refers to motor sequencing, i.e., our ability to organize movements into a precise temporal and ordinal structure. Motor sequence learning will be the main topic of this thesis.

The question of how we learn motor sequences spans across many different disciplines, including (cognitive) psychology, neuroscience, motor control theory, and computational modelling. Each of these fields poses a different set of sub-questions, such as '*Under what conditions do we learn best?*', '*How are memories for motor sequences encoded in the brain?*', and '*What are the underlying rules and functions that determine sequence learning?*'. Thus, while a complete answer will ultimately require a synthesis of these different areas of knowledge, this thesis will dive into some of the more specific questions related to the behavioral and neural mechanisms involved in motor sequence learning.

1.1 Measuring motor skill learning

The following section will give a brief introduction into some important concepts regarding the measurement of motor skills in general. Special emphasis will be placed on the importance of discriminating between measurements of learning and those of performance.

1.1.1 Measuring learning versus performance

When assessing motor learning it is important to keep in mind that we cannot measure learning itself, but that we can only infer that learning has taken place, based on certain changes in behavior (Schmidt 2005). Although the link between learning and improved performance may seem obvious at first, performance measures are not always an accurate index of what has been learned. Learning

refers to the relatively stable change in our capability for a certain skill, whereas performance can be influenced by a number of transient factors such as fatigue, guidance, attention, and motivation (Magill 2014). Therefore, performance measurements on a retention test (i.e., performing the same task again after a delay period) might reflect the permanent changes associated with learning more accurately (Kantak and Winstein 2012).

1.1.2 The contextual Interference effect

One particularly interesting discrepancy between learning and performance has been observed in the contextual interference (CI) effect. CI describes the phenomenon in which practicing two tasks (A and B) in a random order (e.g., ABBABAA) leads to poorer practice performance than a blocked practice schedule (e.g., AAABBB). However, when skill retention is evaluated at a later time point, the pattern is reversed and the random practice group shows superior performance (Magill and Hall 1990; Shea and Morgan 1979). This effect has been well documented for various sports skills (Douvis 2005; Hall et al. 1994; Wrisberg and Liu 1991), as well as for manual tasks such as handwriting (Ste-Marie et al. 2004), drawing (Albaret and Thon 1998) and motor sequence learning (Shea and Morgan 1979; Tanaka et al. 2010; Wymbs and Grafton 2009). Yet, despite its wide prevalence, the exact mechanisms of CI are still unclear. One prominent theory of CI suggests that the advantage of a variable practice schedule arises from repeatedly switching between tasks, which requires a frequent reconstruction of motor plans in working memory (Cross et al. 2007; Immink and Wright 1998; Lee and Magill 1983). This effortful and attention-demanding process of constructing motor plans will then eventually lead to more persistent skill representations in long-term memory (Li and Wright 2000). However, this theory cannot explain reports from later studies, where CI has been found for implicit motor tasks, during which participants were unaware of the learning goal and were thus unlikely to construct any motor plans at all (Lin et al. 2013; Song et al. 2015; Song et al. 2012).

1.1.3 Skill transfer

In contrast to retention, which measures performance under exactly the same conditions, motor skill transfer describes how much of an acquired skill can be applied to a new task or context. This can often be a more relevant criterion for real-life skills, where the context for a certain task may not be identical each time. So far, most research has focused on identifying specific properties of positive transfer, i.e., where practicing one skill facilitates performance on another task. Transfer can then be further described as being broad or narrow, referring to either a wide or specific set of tasks and contexts in which the learned skill can be applied (Adams 1987; Schmidt and Lee 2005). However, transfer can also be negative, in the sense that a previous learning experience interferes with a subsequent task and causes a worse performance.

1.2 Motor sequence learning

This section will introduce relevant concepts in the field of motor sequence learning. Different types of sequence knowledge will be discussed, as well as different sequence learning tasks that are commonly used. Finally, an important strategy for organizing complex sequences, i.e., motor chunking, will be described.

1.2.1 A brief justification of the study of button-presses

The reason why sequences of button-presses are so widely used as a model for skill learning lies probably less in their aesthetic appeal, than in their convenience. Sequence performance can easily be quantified in terms of response times (RTs) and error rates and various sequence properties such as difficulty, familiarity, and context can be easily manipulated by changing the sequence content or training paradigm. Another important feature is that the individual elements (key-presses) are relatively simple and stereotyped movements. Therefore, any changes in performance will most likely reflect learning of the abstract sequential relationships between sequence elements, rather than improvements in how individual key presses are executed in terms of their kinematics. Finally, motor sequence tasks also fulfill the most important requirement for cognitive neuroscience research: they cause relatively little head movement. This makes them a suitable task for further exploration using neuroimaging and neuro-stimulation techniques.

1.2.2 Implicit versus explicit skills

Implicit knowledge typically arises as a byproduct of performing a task repeatedly and it remains inaccessible to conscious control. In sequence learning, implicit knowledge seems to be relatively robust to changes in sequence predictability, but it is easily affected by superficial changes to irrelevant features of the task context (Abrahamse and Verwey 2008; Jiménez et al. 2006). On the other hand, explicit knowledge allows the learner to actively generate and test hypotheses about the sequence structure (e.g., expecting a certain element to be next). It can tolerate changes from the exact practice conditions, but is more sensitive to changes in sequence reliability (Jiménez et al. 2006). Finally, the consolidation of explicit sequence knowledge seems to be more dependent on sleep than that of implicit knowledge (Doyon et al. 2009; Press et al. 2005).

1.2.3 The serial response time (SRT) task

The serial response time (SRT) task, as described by Nissen and Bullemer (1987), has been one of the most widely used sequence learning tasks over the past 30 years. In its basic configuration, it is a four-choice response time (RT) task, in which participants continuously respond to the location of a visual cue by pressing a corresponding button. After each response a new cue will appear, typically with a fixed response-to-stimulus interval (RSI). If, unbeknownst to the participants, the cues appear in a sequential order participants will eventually learn the sequence structure or its

underlying rules. Learning in this case is typically demonstrated by an increase in RTs (and/or errors) when after some time the order of the cues is randomized or shifts to a different sequence.

One of the key features of the SRT task is thus that participants are not instructed to learn any sequence regularities, i.e., learning is incidental. Often, learning is referred to as being implicit, because participants tend to show little to no awareness of the sequential order, especially for short or absent RSIs and/or complex sequence structures. Questions as to whether sequence knowledge in the SRT task is purely implicit and how this could be demonstrated have generated much interesting discussion (see e.g., Curran 2001; Destrebecqz and Cleeremans 2001; Shanks and St. John 1994), but are outside the scope of this thesis. Similarly, many studies have tried to determine whether learning in the SRT task is based on forming associations between successive stimulus features (stimulus-based learning), response features (response-based learning), or response-to-stimulus couplings (response effect learning) (for reviews, see: Abrahamse et al. 2010; Schwarb and Schumacher 2012). Depending on which of these associations is most relevant or informative for a given task, sequence knowledge may be encoded more strongly in either perceptual or motor domains. Again, it is beyond the scope of this thesis to delineate the individual contribution of each of these domains. Throughout this thesis, the term *sequence knowledge* will generally be used without reference to any specific learning modality.

1.2.4 Other sequencing tasks

A number of different sequencing tasks have been developed to address questions that might be more difficult to tackle with SRT-like tasks. Often, these tasks involve a fixed number of elements that are performed as one discrete sequence and participants receive explicit instructions to remember the sequence structure. Different sequence identities can be cued e.g., by different symbols, which makes it possible to compare times for sequence initiation and execution between sequences of different lengths and structural properties (Rosenbaum et al. 1984).

Another task, the discrete sequence production (DSP) task, is similar to the SRT task in that participants have to respond to visually presented cues by pressing spatially corresponding keys. However, in the DSP task, a sequence is performed as one discrete set of responses (usually only 2-7 elements) and after substantial practice the entire sequence can be cued by its first element (Abrahamse et al. 2010; Verwey 2001). As a consequence, performance in the DSP task is typically divided into three processing phases: 1) an initiation phase, which is characterized by a long RT for that first key-press and involves the selection and preparation of the sequence, 2) an execution phase, during which RTs for subsequent key-presses are very fast because they were already selected and prepared during initiation, and 3) a concatenation phase, which is characterized by a slower response after ca. 3-4 elements, indicating the transition to and preparation of a new motor chunk (see section 1.2.5 on motor chunking). Thus, while SRT tasks generally encourage externally guided control and sequence knowledge is associative in nature, discrete sequencing tasks promote

the development of internally guided control and the coding and preparation of grouped responses in the form of motor chunks.

1.2.5 Motor sequence chunking

Motor sequence chunking refers to the process by which several movement elements are grouped together into one larger unit. A motor chunk is characterized by a longer RT for its initiation (reflecting higher processing demands for the selection and planning of the upcoming responses) and short RTs for the remaining within-chunk elements (reflecting the mere execution of already planned elements) (Rosenbaum et al. 1983; Verwey 1996).

In many everyday life tasks chunking is an important strategy for organizing complex sequences into more manageable and less demanding units. For example, when practicing a dance it is natural to first group individual steps together and to then concatenate these sub-sequences into one larger sequence. In motor sequence learning tasks, chunk boundaries can be induced by salient sequence structures such as repetitions or inversions (Koch and Hoffmann 2000), stimulus colors (Jiménez et al. 2011), and insertion of a temporal delay in the response-to-stimulus interval (Stadler 1993). However, chunking patterns also develop in the absence of any salient or externally imposed segmentation structures. Interestingly, these spontaneous grouping patterns are highly idiosyncratic, with different chunk sizes and chunk points for different individuals (Kennerley et al. 2004; Ruitenberg et al. 2014a; Sakai et al. 2003).

1.3 Neural correlates of motor sequence learning

Given our vast repertoire of movements and of ways to interact with our environment it is not surprising that a large portion of the brain is devoted to movement. While the exact functions of the individual areas and their mutual interactions are still somewhat debated, several general organizational principles have emerged. One such principle is the cortical rostro-caudal gradient for cognitive to motor functions (i.e., with respect to the central sulcus, rostral areas are involved in the more high-level cognitive aspects of movement planning, whereas more caudal areas code for more movement related properties) (Badre and D'Esposito 2009). Another such principle is the medio-lateral organization of internally to externally guided movements (i.e., medial areas appear to be more strongly involved in selecting movements based on memory, while more lateral areas seem to play a larger role when selecting movements based on external context, such as visual cues) (Koechlin et al. 2000). The following sections will give a brief overview of the individual subcortical and cortical motor areas, with an emphasis on their possible function in motor sequence learning.

1.3.1 Subcortical areas

1.3.1.1 Basal ganglia

The basal ganglia comprise a set of subcortical nuclei that play an important role in motor learning, skill retention, and action selection. More specifically, the dorsal striatum (i.e., caudate nucleus and putamen) forms, together with its cortical input from primary motor cortex (M1), premotor cortex (PMC), supplementary motor cortex (SMA) and pre-supplementary motor cortex (preSMA), a cortico-striatal motor circuit (Doyon et al. 2003; Hikosaka et al. 2002). Since this circuit remains activated even when participants reach asymptotic skill performance levels, it has been suggested that the striatum is involved in the long-term retention of well-learned motor sequences (Doyon et al. 2009; Lehéricy et al. 2005). Doyon and colleagues pointed out that during sequence learning, activity shifts from the anterior (associative) putamen during early stages, toward the posteroventral (sensorimotor) putamen during later stages (Doyon and Benali 2005; Doyon et al. 2003). Moreover, increased activity specifically during the planning phase of self-generated movements has been found in the anterior putamen (Boecker et al. 2008; Elsinger et al. 2006).

1.3.1.2 Cerebellum

The cerebellum is particularly important for motor control. It is assumed to instantiate the internal model, i.e., the relationship between cortical motor plans (efference copy) and the anticipated sensory consequences (Miall 2010; Shadmehr and Krakauer 2008; Wolpert et al. 1998). A discrepancy between the predicted and the actual sensory consequences of an action gives rise to an error signal, which can then be used to guide learning. In contrast to the long-term involvement of the striatum, a number of studies reported that global cerebellar activity decreases with the progression of motor learning (Doyon et al. 2002; Floyer-Lea and Matthews 2005; Grafton et al. 2008; Penhune and Doyon 2005; Steele and Penhune 2010).

1.3.2 Cortical areas

1.3.2.1 Primary motor cortex (M1)

Although M1's involvement in motor learning is predominantly at the level of motor execution, it has been implicated in the use-dependent acquisition and storage of motor skills (Classen et al. 1998; Galea and Celnik 2009; Hadipour-Niktarash et al. 2007; Zhang et al. 2011). In particular, the formation of muscle synergies has been proposed as a mechanism to improve performance speed and precision (Krakauer and Mazzoni 2011; Penhune and Steele 2012; Shmuelof and Krakauer 2011). Moreover, single cell recording studies have found neurons in M1 that show preferential activity for the transition between specific movements, suggesting a possible role of M1 in the storage of sequential motor skills (Matsuzaka et al. 2007).

1.3.2.2 Premotor cortex (PMC)

The lateral premotor cortex (PMC) can be divided into a dorsal (dPMC) and a ventral (vPMC) part. While the vPMC has been predominantly associated with the control of hand movements during reaching and grasping (Chouinard and Paus 2006; Ehrsson et al. 2001; Rizzolatti et al. 2002), the dPMC seems to be more strongly involved in response selection based on (visual) cues (Kalaska and Crammond 1995; Picton et al. 2007; Rushworth et al. 2003). The dPMC has also been implicated in coding for spatial and sequential aspects of motor selection, although the relative contributions of spatial and sequential components are often difficult to disentangle if sequences are represented by different spatial locations of the cue (Schubotz and von Cramon 2003). In SRT-like tasks, the dPMC is activated bilaterally, even when the task is performed only with the right hand (Hardwick et al. 2013). Activity in dPMC has even been observed in the absence of hand movements, when participants anticipated the spatial positions of sequential stimuli (Schubotz and von Cramon 2001; Shulman et al. 1999).

1.3.2.3 Supplementary motor area (SMA)

The medial premotor cortex, generally referred to as supplementary motor area (SMA), can be divided into two distinct areas, the rostral preSMA and the caudal SMA (also called SMA-proper). The areas have different physiological and anatomical connectivity profiles and one important distinction is that the SMA, but not the preSMA, has direct reciprocal connections with M1 and also sends direct corticospinal efferences (Picard and Strick 1996). In non-human primates, the SMA has been shown to contain neurons that are sensitive to the sequential context of individual movements (Tanji and Shima 1994). In humans, the SMA has been implicated in the self-initiation of voluntary movements (Deecke and Kornhuber 1978; Hoffstaedter et al. 2013) and the learning of sequential motor tasks (Hardwick et al. 2013; Wiestler and Diedrichsen 2013; Wymbs and Grafton 2015). Temporary disruption of SMA activity has been shown to interfere with the organization of upcoming motor sequence elements (Gerloff et al. 1997).

1.3.2.4 Pre-supplementary motor area (preSMA)

The preSMA is involved in the more cognitive aspects of motor planning and also in non-motor cognitive processes (Leek and Johnston 2009; Tanaka et al. 2005). Individual neurons in the preSMA of non-human primates have been found to change their firing pattern over the course of motor sequence learning. As sequences became more familiar, activity became restricted to the initiation of a chunk (Nakamura et al. 1998). Moreover, Shima and Tanji (2000) showed that while neurons in both SMA and preSMA are involved in motor sequencing, SMA neurons were more selective for the temporal interval between movements, while neurons in preSMA represented the ordinal rank-order of movements. A similar role of preSMA in motor sequencing and chunking tasks has been found in humans (Hikosaka et al. 1996b; Kennerley et al. 2004; Sakai et al. 1999; Sakai et al. 1998). For example, Kennerley et al. (2004) reported that stimulation of the preSMA

disrupted motor performance when it was given at the beginning, but not in the middle, of a motor chunk. Yet, the human preSMA has been implicated in a number of functions, some of which might represent components of chunking but not chunking per se. Particularly its involvement in movement inhibition (Chen et al. 2009; Obeso et al. 2013; Picazio et al. 2014), task switching (Roberts and Husain 2015; Rushworth et al. 2002), and action reprogramming (Mars et al. 2009) suggests that the preSMA's role in chunking might rather reflect a common function of suppressing an ongoing movement and switching towards a new action.

Finally, the preSMA is also the most popular brain area in terms of publication preferences (for an entertainig analysis of publication frequency based on reported peak activity, including a regression on impact factor, see Behrens et al. 2013).

1.4 The challenge of interpreting activations

In a meta-analysis, Hardwick et al. (2013) identified brain areas that showed consistent activity increases for motor learning tasks across 70 different neuroimaging studies. A sub-analysis specifically for motor sequence learning paradigms, i.e., SRT-like tasks (35 studies), revealed consistent activations in bilateral dPMC, left M1, SMA, preSMA, left superior parietal lobe (SPL), left thalamus and right cerebellum (see **Figure 1**). Although these findings nicely reflect and summarize the contributions of the individual motor areas, it can be difficult to compare neuroimaging studies on motor learning. A major difficulty is how to interpret changes in activity over time. On the one hand, one might argue that a learned motor sequence should recruit more neural activity in areas that represent the sequential content. On the other hand, learning might lead to increased neural efficiency and therefore one could expect to find decreased activity for trained sequences (Wiestler and Diedrichsen 2013; Wymbs and Grafton 2015). Therefore, a more suitable approach to investigating the neural mechanisms behind motor sequence learning would be to ask which properties of a motor sequence (e.g., its content or its familiarity) are represented in a given brain area, rather than asking which brain areas change their activity level over the course of learning.

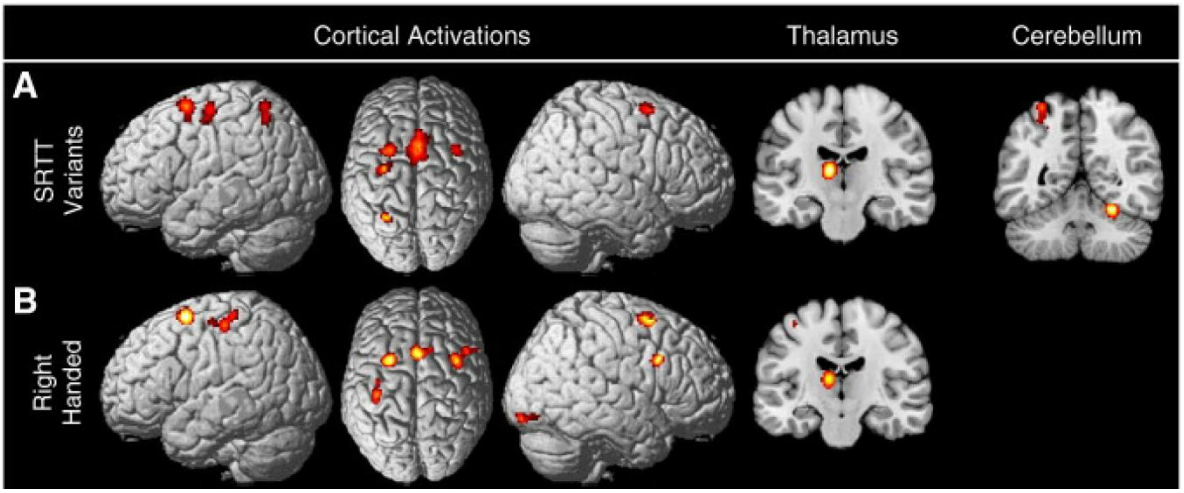


Figure 1. Consistently activated brain areas in a meta-analysis of 35 neuroimaging studies that used SRT-like tasks. **(A)** Areas that showed consistent activity across various variants of SRT-like tasks. **(B)** Areas that were activated in a subanalysis that included only studies (n = 19) with SRT-like tasks that were performed with the right hand. Figure adapted from Hardwick et al. (2013) and reprinted with permission.

2 AIMS

The general aim of this thesis was to investigate behavioral aspects of motor sequence transfer, as well as to characterize the neural correlates of different aspects of motor sequence performance.

- The aims of **Study I** were to

    1) Investigate the effect of practice schedule on motor sequence transfer
    2) Investigate whether sequence-specific knowledge can transfer to an unfamiliar sequence context

    We predicted that, in line with the known benefits of contextual interference in many other tasks, a variable practice schedule would lead to greater skill transfer than constant practice of the same sequence. Further, we expected to find that sequence-specific knowledge can transfer to overlapping sub-sequences that are embedded within a different sequence context.

- The aims of **Study II** were to

    1) Investigate how different motor sequence features, such as familiarity (trained or novel) and structural sequence similarity (similar or different) are represented in cortical activity patterns
    2) Investigate how the neural representations of motor sequences relate to motor performance

    We expected that, especially for trained sequences, structurally similar sequences would recruit similar populations of neurons and would therefore show more similar neural activity patterns. In addition, we expected that differences in sequence performance might contribute to differences in neural representations.

- The aim of **Study III** was to investigate the relative contribution of the pre-SMA to motor chunking and hand switching.

    We hypothesized that if the pre-SMA is necessary for sequence chunking, TMS over this area would delay responses at the initiation of a new chunk (but would not affect later responses within a chunk). If, on the other hand, the pre-SMA is not necessarily involved in chunking per se, but rather in hand switching, we would expect TMS to delay responses in all conditions that require a hand switch, independent of whether this is at the start of a new chunk or within a continuing chunk.

3 METHODOLOGICAL APPROACH

This section will provide an overview of the experimental paradigms and methodological procedures that were used in the studies of this thesis. The tasks, procedures and measurements will be briefly introduced and, where appropriate, the rationale behind their choice will be presented together with a discussion of their specific strengths and limitations. Detailed descriptions of the methods can be found in the individual articles. More general limitations will be addressed in the Discussion section.

A crucial element of all studies in this thesis was the use of various motor sequencing tasks. We applied different modulations of how the sequences were constructed, practiced, and tested to evaluate the specific research questions of each study. Since the specific design of the sequencing task can have a large impact on the measured outcome, more emphasis will be placed on describing the behavioral tasks. Although behavioral tasks can, to a certain extent, reveal underlying neural mechanisms, we also used neuroimaging (fMRI) and brain stimulation (TMS) techniques to explore the neural correlates of motor sequences and chunking. Methodological considerations for each of these techniques will be discussed in relation to the respective goals of the studies.

3.1 Participants

All participants in the studies were right-handed young adults and had no neurological or other problems related to hand or finger movements. Participants in **Study I** (n = 60) and **Study II** (n= 45) were recruited from the Stockholm area, using flyers and a Swedish website for research volunteers (www.studentkaninen.se). Participants in **Study III** (n = 18) were recruited from a database of healthy volunteers for clinical trials at the National Institutes of Health (NIH) at the Bethesda, MD campus in the United States. For studies II and III volunteers were screened to fulfil all safety guidelines for participation in MRI and TMS experiments, respectively. All participants gave written informed consent before the start of the experiment. All studies were approved by the Regional Ethical Review Board in Stockholm, Sweden (**Studies I** and **II**) or by the Neuroscience Institutional Review Board of the NIH in Bethesda, MD, USA (**Study III**) and conformed to the ethical guidelines of the Declaration of Helsinki.

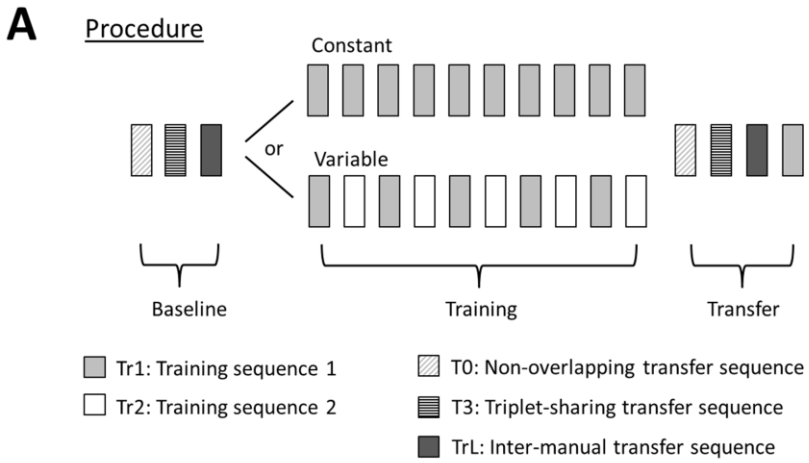
3.2 Equipment for behavioral tasks

All tasks used custom written scripts in E-Prime (Psychological Software Tools, Inc) to present visual stimuli and collect participants' responses. Except for the fMRI part of **Study II**, all stimuli were presented on a standard monitor positioned at a viewing distance of ca. 60 cm and responses were made using designated keys on a regular qwerty keyboard.

3.3 Sequences and tasks

3.3.1 Study I

In this study, participants were divided into two groups that practiced either one ('Constant' group) or two ('Variable' group) sequences for a total of 10 blocks. Each block contained 10 uninterrupted repetitions of the same sequence. Performance on three different transfer sequences was evaluated immediately before and after training (see **Figure 2, A**). All sequences were performed as SRT tasks (Nissen and Bullemer 1987), i.e., a cue would appear in one out of four possible response locations and the participant's goal was to press the corresponding key as quickly as possible. The location of the visual cue always matched the spatial location of the required response and all sequences, except for transfer sequence TrL, were performed with the index to little finger of the right hand.



B

Training Sequences	Transfer Sequences
Tr1: 1 2 4 3 2 3 1 4 (1 3 4) 2	**T0:** 1 2 1 3 2 4 1 4 2 3 4 3
Tr2: 1 2 3 (1 3 4) 2 4 3 2 1 4	**T3:** 1 2 (1 3 4) 3 2 4 1 4 2 3
	TrL: 1 2 4 3 2 3 1 4 1 3 4 2

Figure 2. Experimental procedure and sequential stimuli. **(A)** The experimental procedure consisted of three phases: Baseline, Training, and Transfer. The Baseline phase consisted of one block of each of the three transfer sequences (T0, T3, TrL). The Transfer phase consisted of the same three sequences (presented in the same order as during Baseline) plus one additional block of Tr1 at the end of the phase. Block order during the Baseline phase was randomized across participants and counterbalanced between groups. In the Training phase, the Constant group performed 10 blocks of the Tr1 sequence, while the Variable group alternated between Tr1 and Tr2 blocks. All blocks were separated by 20 s of rest, both within and between the phases. Each block contained 10 uninterrupted sequence repetitions, thus requiring a total of 120 responses (12 sequence elements x 10 repetitions) per block. **(B)** Sequence structure of the training (left) and transfer (right) sequences. The training sequences (Tr1 and Tr2) shared 6 triplets with each other, three of which were also shared with sequence T3 (one example of a shared triplet encircled). None of the training sequences shared any triplets with sequence T0.

One methodological challenge in comparing performance between different sequences is to avoid possible confounds due to differences in saliency or difficulty. Since RT improvements may reflect knowledge of both complex sequential structures and simpler statistical regularities (e.g., individual item or transition frequencies), it is important to disentangle the sequence order from statistical properties. Therefore, we used complex second-order conditional sequences (see Reed and Johnson 1994), in which the identity of any given sequence element could only be predicted based on the preceding two elements, but not on one element alone. In addition, all sequences were matched on the following criteria: length, individual item frequency, bigram frequency, reversal frequency, rate of full coverage, and rate of full transition usage (see **Table 1** for details).

Table 1. Statistical properties that all sequences were matched on.

Sequence Property	Explanation/ Example	Amount
Length	Total number of elements	12
Individual item frequency	1, 2, 3, 4	0.25
Bigram frequency	12, 13, 14, 21, 23, 24, 31, 32, 34, 41, 42, 43	0.083
Reversal frequency	121	0.25
Rate of full coverage	Average number of elements encountered before each element has occurred at least once	5.08
Rate of full transition usage	Average number of elements encountered before each possible transition has occurred at least once	13

Moreover, the sequences were designed to address different forms of skill transfer, i.e., task-general, inter-manual, and sequence specific transfer. The sequence structure of all sequences is shown in **Figure 2, B**. By choosing deterministic, rather than random control sequences, we were able to control precisely the amount of sequence overlap. Sequence T0 shared no triplets with either training sequence (Tr1 or Tr2) and was used to evaluate sequence non-specific transfer. Sequence T3 shared the same three triplets (134, 231, and 432) with both training sequences and thus served to evaluate sequence-specific transfer. Finally, sequence TrL was identical to the primary training sequence (Tr1) and was used to examine inter-manual transfer, while keeping the order of visual stimulus locations and response locations intact.

Since we were interested in transfer of implicit, rather than explicit, sequence knowledge we used an SRT task with a response-to-stimulus interval of 0 ms, i.e., the next stimulus appeared immediately after a correct response (see Destrebecqz and Cleeremans 2001). Moreover, participants were told that they participated in a reaction time experiment and were not informed about the sequential nature of the stimuli. However, it is important to note that it is notoriously difficult to demonstrate that sequence skills on an SRT task are *purely* implicit (Abrahamse et al. 2010; Wilkinson and Shanks 2004) and we cannot exclude the possibility that participants might

have developed at least some explicit sequence knowledge. Nevertheless, since it was not in the scope of this study to differentiate between the relative contributions of implicit and explicit sequence knowledge to sequence transfer, and since previous studies found no relation between explicit sequence knowledge and the amount of transfer (Sanchez et al. 2015; Song et al. 2012), we do not think that the development of some explicit sequence knowledge would have been problematic for this study.

3.3.2 Study II

In Study II, we again made use of an SRT-like task, but this time participants were explicitly informed about the deterministic order of the stimuli and were told to remember the order of the three training sequences.

3.3.2.1 Sequences

Again, the sequences were constructed according to specific criteria. All sequences were 8 elements long and contained 2 instances of each digit. There were two matched sets of sequences (see **Figure 3**). One group of participants practiced Set 1 and the other group practiced Set 2. For both groups, the non-practiced set was introduced as novel sequences during fMRI scanning. Each set contained two similar sequences, which consisted of the same sub-sequences (chunks) ordered differently and one different sequence, which was based on entirely different chunks. No chunks were shared between the two sets.

Figure 3. Similarity structure of the two sets of sequences. Both sets contained two similar sequences, A, B and X, Y, and one different sequence, C and Z, respectively. The similar sequences consisted of the same sub-sequences (marked in the same color), but the order of the first and last sub-sequence was reversed. The different sequence did not share any sub-sequences with either of the other sequences. No sub-sequences were shared between sets.

3.3.2.2 Practice tasks

Participants were divided into three equally large groups (n = 15, each). Two groups practiced three sequences on two consecutive days for ca. 1 h per day and then underwent fMRI scanning while being tested on the trained sequences, as well as on three novel sequences. The third group

served as a control group and performed the same sequences inside the scanner as the other groups, but without prior sequence training.

On each day, the training consisted of 4 slightly different tasks. In the first task, an entire sequence was divided into 3 parts, which were all displayed on the screen simultaneously (see **Figure 4, A**). The sequence was displayed in 3 parts to promote chunking into the desired structure of 3, 2, and 3 elements. Each part was to be read from top to bottom and each row cued one sequence element (i.e., the correct response key corresponded to the location of the yellow square). Each of the 3 training sequences was labeled with a different letter. Participants were asked to practice typing each sequence for one minute, followed by typing it once from memory. Each sequence was performed 3 times. After that, participants could rest for one minute while all three sequences were displayed below each other on the screen (**Figure 4, B**). This practice phase was different from standard SRT or DSP tasks, but it allowed us to present the sequences in the desired chunking format, thereby increasing the probability that participants would recognize the identical chunks in the similar sequences.

From the second task onward, all sequence elements were presented one cue at a time, i.e., similar to a classic SRT-task. In the second task, participants had to synchronize their key-presses to the onset of the cue (yellow square), which remained visible until the onset of the next cue, 900 ms later (**Figure 4, C**). Each sequence was cued with its corresponding letter at the start of each trial.

The third task was similar to the previous one, except that the sequence cue appeared only briefly (for 200 ms) and after that the squares stayed empty for the remaining 700 ms. The final training task was the same as the previous one, except that no sequence label was shown at the start of a trial. Further, one novel sequence was presented amongst the training sequences. A novel sequence was introduced to familiarize participants with the procedure during scanning, where three novel sequences would be added. The novel sequence used during training was different from all sequences used during the fMRI task in that it had no chunks in common with the sequences of either set.

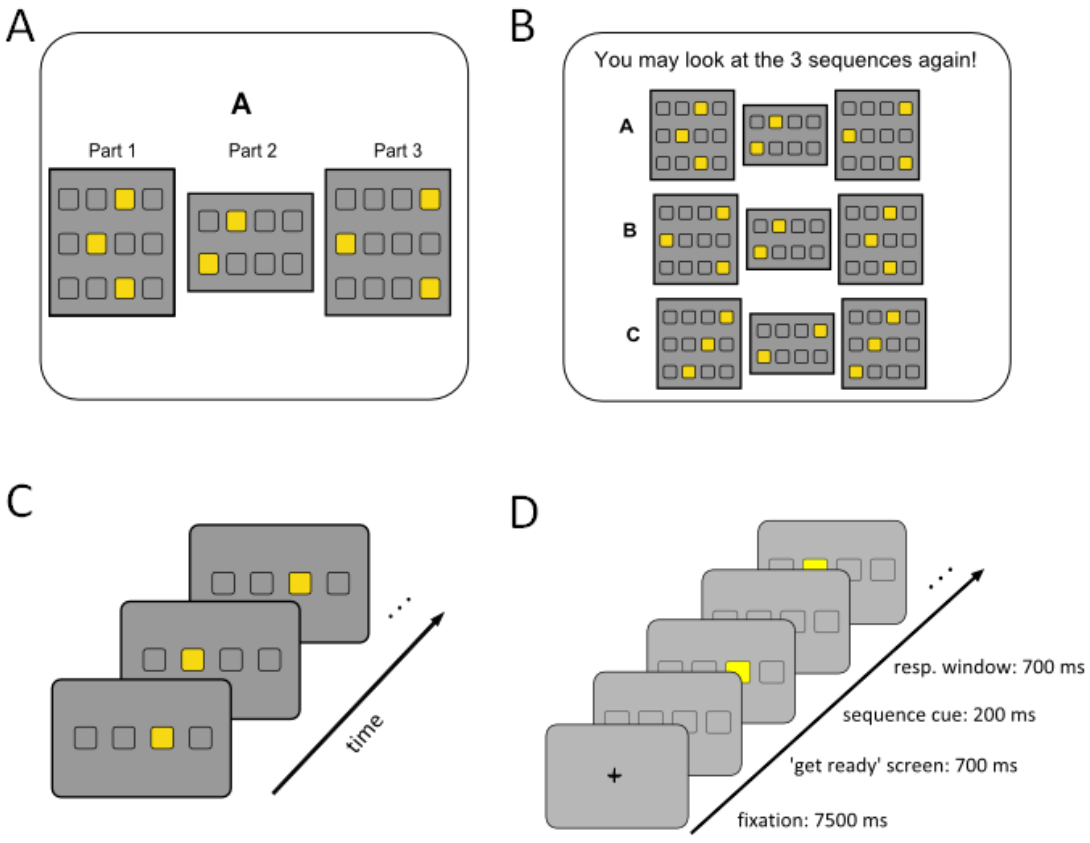


Figure 4. Training paradigm. **(A)** Example stimulus from the first part of the training. The response location for each sequence element was cued by the yellow box. The entire sequence was displayed on the screen and segmented into three parts. **(B)** During rest, participants were encouraged to visually study all three sequences again. **(C)** In the later practice phases only one sequence element at a time was displayed and participants were encouraged to make use of their sequence knowledge to press the corresponding key as quickly as possible. **(D)** Stimulus presentation during fMRI scanning. Each trial started with 7500 ms of fixation, followed by 700 ms of the stimulus without a sequence cue (empty squares) to indicate that the first sequence cue would appear soon. Sequence cues (yellow squares) were shown for 200 ms followed by a fixed response window of 700 ms.

3.3.2.3 fMRI task

During the fMRI session the participants' task was the same as in the final training part, except that three novel sequences were added. For both trained and novel sequences, a trial started with 7500 ms of fixation and 700 ms of a 'get ready' stimulus (empty squares) (**Figure 4, D**). Each sequence element (cue) was shown for 200 ms, followed by a response window of 700 ms during which all squares remained empty. This fixed inter-stimulus-interval of 900 ms allowed us to keep the total trial length and the number and frequency of responses equal for trained and novel sequences. Thereby, it was possible to avoid possible confounds due to rate effects (i.e., changes in the hemodynamic response as a function of movement frequency) (Deiber et al. 1999; Lutz et al. 2005).

Participants performed 6 runs, each containing 12 sequence trials. After 3 runs, participants were allowed to briefly rest, while an anatomical (T1-weighted) MRI was collected. Each run contained two trials of each sequence. The trial order within a run was mirrored at the midpoint of the run, so that if e.g., the first 6 trials within a run were in the order A, B, C, X, Y, Z, the remaining 6 trials would be in the reversed order Z, Y, X, C, B, A. The sequence order within runs was counterbalanced across runs, such that across all runs each sequence was presented once at each of the 12 trial positions. Careful counterbalancing of the order of all trials and runs is very important for RSA analysis (see section 3.5). The fMRI signal intensity may vary across both the duration of a single run and across the entire scan session, due to various factors such as scanner drifts, temperature fluctuations, and participants' head motion. Therefore, signals measured from successive trials are likely to be more similar than signals from distant trials. Thus, when correlating activity patterns across different halves of the data, it is crucial that the images within each half are matched with respect to their relative position within a run and session.

Inside the scanner we used an MR-compatible button-box to collect participants' responses. Visual stimuli were projected on a screen at the back of the scanner, which participants could view through a mirror mounted on the head coil.

3.3.3 Study III

Since the goal of **Study III** was to dissociate chunking from hand-switching, we designed bimanual sequences that contained all possible combinations of hand-switches (i.e., switch towards or away from the right hand, or no hand-switch) and chunk-boundaries (i.e., within or between chunks).

3.3.3.1 Sequences

In total, we designed 16 sequences, which were all based on different combinations of the same four chunks. **Figure 5** shows an example sequence with all possible chunk and switch combinations outlined. In addition, we constructed the sequences such that the involved digits at each type of chunk and switch transition were matched as much as possible. Further, we avoided any direct repetition of the same digit.

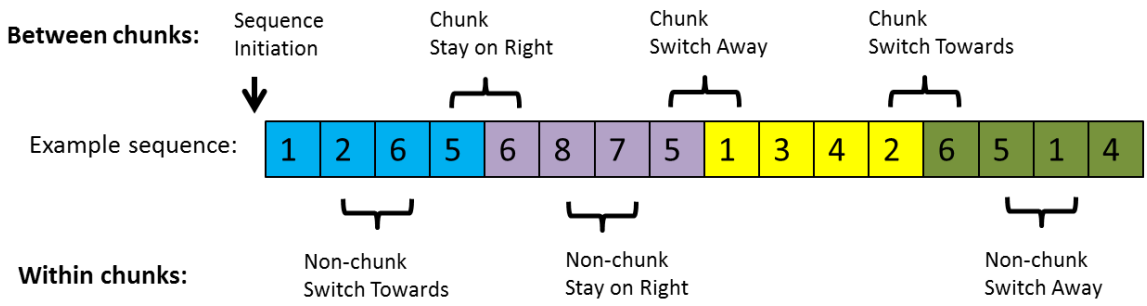


Figure 5. Sequence design. The sequences and sub-sequences were designed to contain the same hand transitions between-chunks as within-chunks. The colors represent different sub-sequences (chunks) and the numbers represent the finger to be used (1-4 for the little to index finger of the left hand, 5-8 for the index to little finger of the right hand). The sequence shows the 7 transition points of interest, i.e., points at which RTs were evaluated with and without TMS. There were 2 chunk boundary conditions (between- or within-chunk), each containing the same 3 hand-switch conditions (switch towards the right hand, switch away from the right hand, and stay on the right hand), plus the sequence initiation condition.

We used many (16) different target sequences, rather than just one or a few, for several reasons. First, varying the chunk-order on a trial-by-trial basis, made it unlikely that participants would fuse chunks together or would develop other chunking patterns than the intended 4x4 structure. Second, if a given chunk or switch transition always occurs at the same sequence position, as would be the case in a single sequence, any effects on that transition type would be confounded with possible effects due to its ordinal position within the sequence. Therefore, we decided to use many different chunk orders, so that we could counterbalance the relative position of the chunk/switch transitions.

3.3.3.2 Task

The experiment consisted of a practice phase, during which the four sub-sequences were practiced in two different tasks, and a test phase, during which the sub-sequences had to be concatenated into the full sequences. During all practice trials, the identity of each sub-sequence was cued by the background color of the screen (blue, purple, yellow, or green). In the first training task, each sequence element was cued by the position and number of a lit-up square that corresponded to the correct response location (**Figure 6, A**). In the second task, the placeholder squares did not light up, and sequence identity was only cued by the background color of the screen. Participants had to type the sequence from memory (**Figure 6, B**). In the testing phase, the order of the full sequence was cued by four colored boxes on the screen, where the color of each box represented one of the previously trained sub-sequences (**Figure 6, C**). Participants were instructed to type the full sequence as quickly as possible, without making errors. Incorrect responses let to an immediate trial abortion. This was necessary to ensure that we would collect an equal number of correct trials for each condition and to avoid unnecessary TMS stimulation for invalid trials.

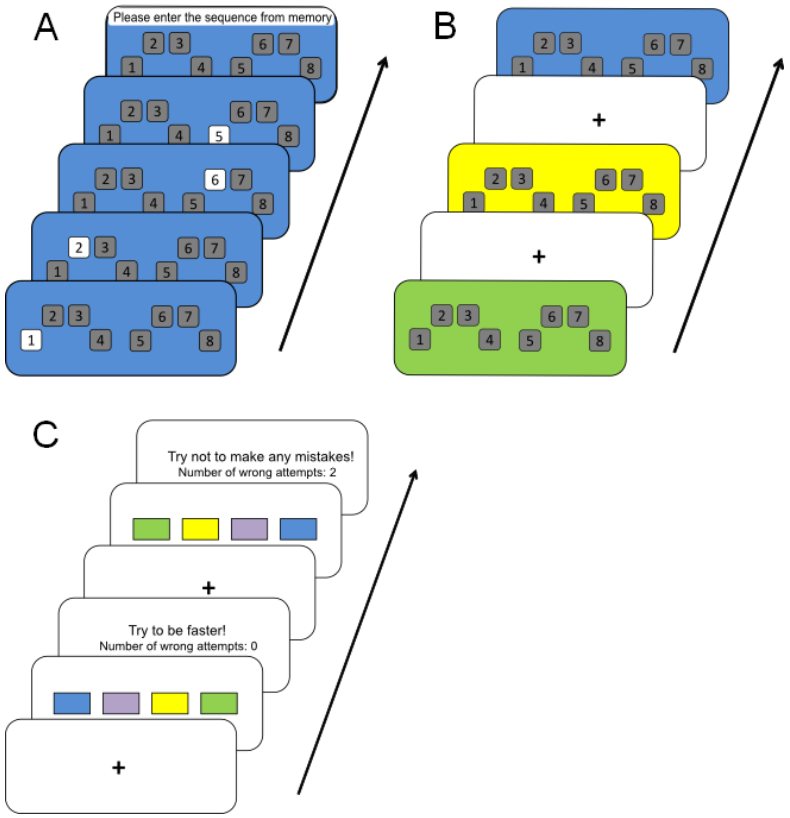


Figure 6. Training and testing tasks**.** During training, participants practiced each sub-sequence separately. **(A)** In the initial training phase, participants saw 8 grey boxes representing the response keys for the little to index finger of the left hand (boxes 1-4) and for the index to little finger of the right hand (boxes 5-8). Each sub-sequence corresponded to a different color that was represented by the background color of the screen (blue, purple, green, or yellow). The sequence order was indicated by the order in which the boxes lit up white. Participants responded to the lit-up boxes by pressing the corresponding key. Each sub-sequence was 4 key-presses long. After 3 repetitions of responding to the lit-up boxes of a sub-sequence, participants typed the sequence once from memory. **(B)** In the second training phase, participants typed the sub-sequence corresponding to each background color from memory. The sub-sequences were presented in random order. **(C)** During testing, participants first saw a fixation cross, followed by the sequence cue consisting of 4 horizontally aligned boxes. The colors of the boxes corresponded to the previously memorized sub-sequences and were to be 'read' from left to right. No feedback was provided during sequence performance, but errors caused the trial to be aborted and wrong trials had to be repeated until they were error-free. Correct trials were followed by performance feedback, showing the number of incorrect attempts and either an encouragement not to make any mistakes (if they had incorrect attempts), to be faster (if they had no incorrect attempts, but were slower than the mean + 2 SD of their previous 3 trials), or a "Well done!" message. The sequence order, i.e., the order of the colored rectangles, varied pseudo-randomly between trials. There were 8 blocks, each containing 21 trials.

During the testing session, TMS was applied over either left M1 or left preSMA (order varied pseudo-randomly across blocks). Within any given trial, TMS could be delivered either at sequence initiation (120 ms after the onset of the sequence cue), at any of the six chunk/switch transitions of interest (1-2 ms after the previous key press), or not at all (control trials).

3.4 fMRI

For **Study II**, MRI data was collected on a 3T GE scanner with an 8-channel head coil, located at the MR-center in Stockholm, Sweden. We used fMRI to non-invasively measure neural activity in relation to motor sequence performance.

3.4.1 The fMRI signal

An important point to keep in mind when discussing fMRI studies is the nature of the fMRI signal, the blood oxygenation level dependent (BOLD) response. When neurons become more active, their metabolic demands (for glucose and oxygen) increase. The vascular response to that is an increase in cerebral blood flow and blood volume, resulting in a net increase in the ratio of oxygenated versus deoxygenated hemoglobin. Because of the different magnetic properties of oxygenated (diamagnetic) and deoxygenated (paramagnetic, i.e., perturbing the main magnetic field) blood, an increase in neural activity will therefore increase the MRI signal (Norris 2006). In addition to being an indirect measure of neuronal activity, the BOLD response also has a considerable delay of ca. 5 seconds before it reaches its peak (de Zwart et al. 2005). Further, simultaneous intracortical and fMRI recordings have shown that the BOLD response is more closely related to the input and intracortical processing of a given brain region, than to its spiking output (Logothetis et al. 2001; Logothetis and Wandell 2004).

Another limitation of fMRI, which is more specific to its use in our study, is its relatively poor temporal resolution. Since the images of a full volume of the brain are typically acquired within 2-3 s (2.5 s in our study), it was not possible to track the signal changes associated to individual button presses, which occurred at a rate of ca. 0.9 s. Yet, compared to other non-invasive neuroimaging techniques, fMRI has the best spatial resolution (3 mm isotropic in our study).

3.4.2 fMRI analysis

In most standard processing pipelines for univariate fMRI analysis, the data are first preprocessed by applying head-motion correction (i.e., spatially realigning the image series to a reference image), spatial smoothing (e.g., with an 8 mm FWHM Gaussian kernel), and spatial normalization to a template brain (e.g., to the MNI average template). After that, the data are commonly analyzed using a univariate general linear model (GLM). In the GLM, the time-course of the measured neural activity is explained by a linear combination of independent explanatory variables (experimental conditions), plus noise. The experimental conditions are modeled by their onset and duration, and are convolved with the hemodynamic response function to account for the delay in the BOLD response. Additional nuisance regressors (e.g., the time course of head motion parameters) can be added to account for additional variance in the data. The GLM then yields one coefficient for each explanatory variable, indicating how strongly the temporal activity profile in the

present voxel followed the idealized time course of that condition. The same model is estimated independently for each voxel.

As a consequence, statistical inference (typically t-tests) has to be carried out in every individual voxel, resulting in a multiple comparisons problem (i.e., since every test in itself carries typically a 5 % risk of a false positive, repeating it in ≈ 100k voxels would mean accepting the risk of a type I error in ≈ 5k voxels). The standard Bonferroni correction for multiple comparisons does not take the spatial auto-correlation of the fMRI data into account and would therefore be too conservative. An alternative method, which is also implemented in the SPM software package (Statistical Parametric Mapping, Wellcome Center for Neuroimaging, University College London, UK), is to apply family-wise error (FWE) correction based on random field theory (Friston 2007). This method estimates the number of independent resolution elements (*resels*) by taking the spatial smoothness of the data into account. The required p-value for significance is then divided by the number of resels, which is much smaller than the number of individual voxels, resulting in a less conservative threshold (Friston 2007; Poline et al. 1997; Worsley et al. 1996).

Despite its popularity, this univariate analysis approach has several drawbacks. One major disadvantage is that by looking for brain areas that show a strong increase in activity on average, one may overlook valuable information that resides in the joint spatial activity pattern of several, more noisy voxels (Kriegeskorte and Bandettini 2007). More recent data analysis frameworks such as multivariate pattern classification and representational similarity analysis (RSA) are able to take the spatially distributed pattern of the fMRI response into account. This shift in analysis technique does not only lead to greater sensitivity, but more importantly, it allows researchers to change their research questions away from "Which brain area is active during task X?" towards "What information is represented in brain area X?" (Haxby et al. 2014; Kriegeskorte and Bandettini 2007). In **Study II** we made use of RSA to compare the neural representations of different motor sequences. This method will be discussed in more detail in the following section.

3.5 Representational similarity analysis (RSA)

RSA takes advantage of the inherently multivariate nature of fMRI data in that it considers the joint activity pattern of all voxels within a given region of interest (ROI). In **Study II** we were interested in how sequences are represented at three different anatomical sites, M1, SMA, and PMC. Therefore, we selected our ROIs based on anatomical masks of these regions, using the WFU PickAtlas (Functional MRI Laboratory, Wake Forest University School of Medicine, NC) as implemented in SPM12. Alternatively, ROIs can be selected based on functional activity maps, as long as the underlying dataset or contrast is independent from the subsequent analysis (otherwise one inflates the risk of false positives due to circular analysis) (see Kriegeskorte et al. 2009). In addition to the anatomical ROIs, we used one functional ROI that included all voxels that were active during the task (i.e., irrespective of sequence type or condition).

Data preprocessing for RSA differs from that for univariate analysis, in that spatial normalization to a template image is typically omitted and spatial smoothing is reduced to a smaller kernel size (e.g., 6 mm FWHM in our study) or not applied at all. The data are then split into halves (e.g., even and odd runs) and a GLM is performed on each half separately. Within a given ROI, one can then extracted the spatial activity pattern for each variable. In our study we extracted the activity patterns by using a t-test for each sequence (e.g., Sequence A – rest). Finally, the activity patterns (e.g., the absolute t-values) of all conditions (sequences) are cross-correlated across the two halves of the data. The resulting correlation matrix can then be visualized to inspect the representational similarity between individual sequences or sequence categories (e.g. trained or novel).

An additional benefit of RSA is the fact that the similarity matrix is a modality-independent abstraction of the activity pattern. Therefore, it can be quantitatively related to measurements in different modalities (Kriegeskorte et al. 2008). This is particularly useful for fMRI studies of motor performance, because behavioral measurements, which are typically collected together with fMRI data, can be related to neural activity patterns. Yet, **Study II** is, to my knowledge, the first one to directly relate similarities in motor performance to similarities in neural representations.

3.6 TMS

In **Study III** we used TMS to temporarily interfere with neural processing in the preSMA while participants were performing motor sequences. During TMS stimulation, a brief and strong electric current is passed through a wire coil, thereby inducing a high-intensity magnetic field. If the coil is placed tangentially over the skull, the magnetic field will travel into the brain and will locally induce a small electrical current via electromagnetic induction (Barker et al. 1985; Hallett 2007). The electrical current changes the membrane potential of the neurons, leading to either depolarization or hyperpolarization, depending on the exact stimulation parameters. A single TMS pulse over M1 will typically lead to depolarization and can, if strong enough, evoke a descending corticospinal volley. The motor-evoked-potential (MEP) can then be measured in the corresponding muscle (Di Lazzaro et al. 1998) using surface electromyography (EMG). The resting motor threshold (RMT) is typically defined as the stimulation intensity that produces MEPs larger than 50 μV in 5 out of 10 successive trials while the hand is at rest (Rossini et al. 1994).

Repetitive TMS protocols such as continuous theta burst stimulation (cTBS, i.e., sets of 3 pulses at 50 Hz that are repeated every 200 ms over 40 s) have been found to have very powerful suppressive effects on MEP amplitude and are therefore well suited to inhibit processing in a certain cortical area (Huang et al. 2005). However, since our goal was to inhibit neural activity at specific points during motor sequence performance, we needed a stimulation protocol with higher temporal precision. Therefore, we used double-pulse TMS (dTMS) with an inter-pulse interval of 40 ms, which had previously been shown to be effective in disrupting local neural processing (Pitcher

2014; Pitcher et al. 2007). We administered both pulses with the same stimulation intensity. Over M1, stimulation intensity was 110% RMT and over preSMA it was 120% RMT. We chose a lower stimulation intensity over M1 to avoid twitching of the hand muscles, which could have interfered with task performance.

To establish the RMT in each subject, we first identified their motor hotspot, i.e., the site over the left M1 that consistently elicited the largest MEPs in the right first dorsal interosseous (FDI) muscle. After that, we determined the RMT as described above. MEPs were recorded using disposable surface Ag-AgCl electrodes, placed in a belly-tendon montage. The location of the preSMA was defined as the point on the left hemisphere just anterior to the vertical line passing through the anterior commissure (AC) and perpendicular to the AC-PC plane (Mayka et al. 2006; Picard and Strick 1996). We used an anatomical MRI scan from each participant, together with Brainsight TMS navigation software (Rogue Research Inc., Cardiff, UK), to identify and mark the target locations for preSMA and M1 in each individual participant. **Figure 7** shows a screenshot of a target area (M1) in Brainsight. The location and corresponding coil position for both M1 and preSMA were marked on a swim-cap that participants wore throughout the experiment. We used a figure-of-eight-shaped coil (70 mm external loop diameter), connected to a Magstim 200^2 stimulator (Magstim, Whitland, Dyfed, UK) that generated monophasic TMS pulses. For M1 stimulation, the coil was positioned with the handle pointing backwards and 45° away from the midline of the skull (Janssen et al. 2015b; Sakai et al. 1997). Over preSMA the coil was oriented in a latero-medial position, with the handle pointing towards the left and 90° away from the midline of the skull (Arai et al. 2012; Janssen et al. 2015a).

Figure 7. Example of the localization of a target area (left M1) using an individual's MRI scan and Brainsight software. *Left:* Localization of the target area on a sagittal slice view. *Right:* Skull-stripped rendering of the brain surface with the calculated location and orientation of the TMS pulse (green cone) and a symbol depicting the orientation of the TMS coil.

The major advantage of TMS is that it is currently the only non-invasive technique that can be used to safely interfere with local brain processing. Two limitations of TMS with respect to its use in **Study III** are that its neurophysiological effects are still not completely understood and that the spatial spread of its effect is difficult to predict. Importantly, its spatial resolution is not only limited by the passive spread of the induced electrical current through brain tissue, but also due to its interaction with, and spread via, white matter fiber tracts (Nele De et al. 2016; Seo et al. 2017). Therefore, since the spread of the stimulation is difficult to predict, one needs to be cautious to ascribe any observed TMS effects exclusively to the brain area over which it was applied.

4 OVERVIEW OF STUDIES AND RESULTS

4.1 Study I

In study I we set out to examine motor sequence transfer and how it can be affected by practice schedule and sequence context. A large body of literature has demonstrated an advantage of variable practice (i.e., alternating between different versions of a task) over blocked practice (i.e., constantly repeating the same task within a block). This so-called contextual interference effect has been found in a variety of motor learning tasks, but its role in incidental sequence learning is less well understood. Therefore, the first aim of this study was to investigate the effect of practice schedule on implicitly learned motor sequences. We predicted that, similar to its effects on other motor tasks, variable practice would lead to greater skill transfer in a motor sequence task. Another open question regarding sequence transfer is its flexibility. Implicit sequence knowledge has been demonstrated to transfer to a new context when the entire familiar sequence was embedded within random elements (Jiménez et al. 2006). However, it remains unclear whether the context of the entire trained sequence is necessary for transfer, or if individual sub-sequences or movement transitions can transfer independently of their original context. Thus, a second aim of this study was to investigate whether skill transfer would be larger for sequences that contain overlapping sub-sequences and whether performance would be specifically improved for familiar sequence transitions.

We randomly assigned participants (n= 60) to one of two training groups. Group 1 practiced the same sequence (Tr1) for 10 blocks in an implicit SRT-like task ('Constant' group). Group 2 alternated between practicing sequence Tr1 and an additional sequence (Tr2), for a total of 10 blocks. Transfer (i.e., pre- to post-training RT differences) was evaluated in three different transfer sequences: task-general transfer was measured in a non-overlapping sequence (T0), sequence-specific transfer was measured in a sequence that shared three triplets with both training sequences (T3), and inter-manual transfer was assessed in a visually identical sequence that was performed with the left hand (TrL).

In each of the three transfer conditions we tested our prediction that variable practice would lead to greater transfer using a repeated-measures GLM with the within-subjects (WS) factor Session (Baseline, Transfer) and the between-subjects (BS) factor Group (Constant, Variable). In line with our prediction, we found that transfer to the T0 sequence was greater after variable practice, as indicated by a significant Session × Group interaction in the predicted direction [$F(1,54) = 3.7$, $p = 0.03$, one-tailed]. However, contrary to our expectation, there was no such effect for the TrL ($p = 0.38$, one-tailed) and the T3 ($p = 0.25$, one-tailed) sequences. All three sequences showed evidence of skill transfer, as confirmed by a significant main-effect of session, i.e., faster RTs at Transfer than at Baseline (T0: $F(1,54) = 39.7$, $p < .001$, TrL: $F(1,52) = 94.31$, $p < .001$; T3: $F(1,54) = 48.56$,

$p < .001$). **Figure 8, A** shows the positive skill transfer, as measured in RT at Baseline – RT at Transfer, for each sequence. Note also the benefit of variable practice for task-general transfer (T0).

The correlation between improvement in T0 and the amount of RT improvement on the training sequence (Tr1) was significant only in the Variable group ($r = 0.47$, $p = 0.01$; **Figure 8, C**), but not in the Constant group ($r = 0.07$; $p = 0.72$; **Figure 8, D**). Similarly, the correlation between T3 and Tr1 improvement scores showed a strong positive trend in the Variable group ($r = 0.37$, $p = 0.055$), but not in the Constant group ($r = -.23$, $p = .24$). Thus, the positive relation between transfer and performance gains during training seems to be specific to variable training.

As expected, we found sequence-specific transfer in addition to task-general transfer. This was indicated by a significant main effect, i.e., larger improvements for T3 than T0, in a rmGLM with the WS-factor Transfer Sequence (T0, T3) and the BS-factor Group (Constant, Variable) [$F(1,54) = 4.06$, $p = .025$, one-tailed]. More importantly, we found that predictable sequence elements (i.e., the last element of shared triplets) showed greater RT improvements than corresponding (same digit) non-predictable elements (**Figure 8, B**). A rmGLM analysis of the Improvement Scores with the WS-factor Element type (T3-Predictable, T3-Non-predictable, T0-Non-predictable), the BS-factor Group (Constant, Variable), and the Element × Group interaction confirmed a significant main effect of Element type [$F(2,107) = 10.0$, $p < .001$, one-tailed], with no effect of Group [$F(1,54) = 2.1$, $p = .15$] or Element type × Group interaction [$F(2,107) = .09$, $p = .91$]. Bonferroni-corrected pairwise comparisons further confirmed that predictable elements in T3 showed greater RT improvements than corresponding non-predictable elements in both T3 [$F(1,54) = 11.8$, $p = .004$] and T0 [$F(1,54) = 16.2$, $p = .001$].

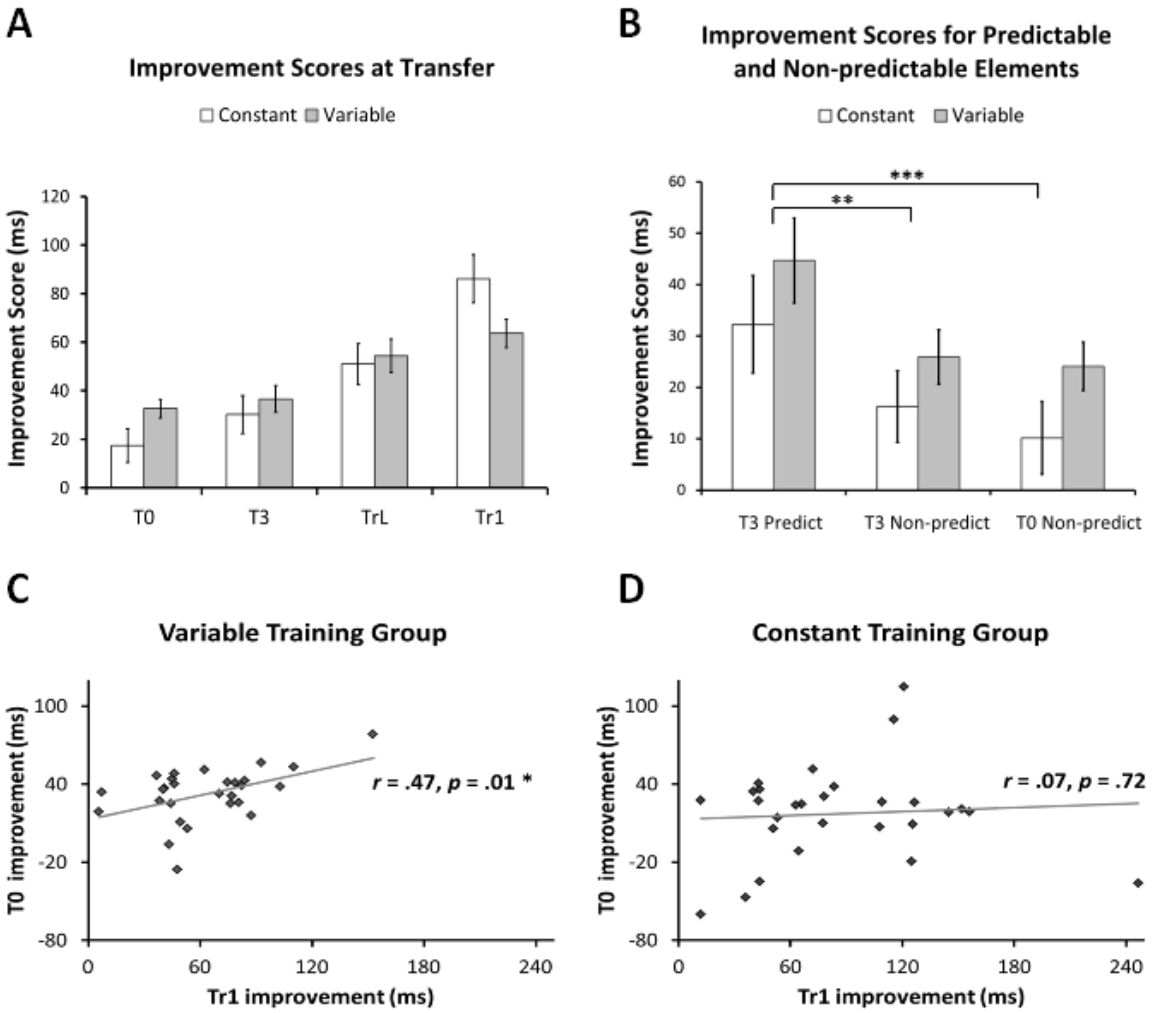


Figure 8. Transfer after constant and variable sequence practice. **(A)** Skill transfer as measured in RT improvement in the non-overlapping (T0), triplet-sharing (T3), left-hand (TrL), and training (Tr1) sequences. **(B)** RT improvements for predictable elements (i.e., average of the last elements in familiar triplets in T3) and corresponding (i.e., same digit) non-predictable elements in T3 and T0. Transfer to predictable elements was larger than to corresponding non-predictable elements in both transfer sequences (T3 and T0). There was no difference between groups and no interaction between group and predictability. **(C)** In the Variable training group, RT improvements in the non-overlapping T0 sequence correlated significantly with improvements on the trained sequence (Tr1). **(D)** In the Constant training group, RT improvements in T0 did not correlate with improvements on the trained sequence. *p = 0.01, **p = 0.004, ***p = 0.001

4.2 Study II

The purpose of **Study II** was to investigate how different motor sequences are represented in the brain. More specifically, we wanted to characterize how different properties of motor sequences, such as their familiarity, similarity, and performance level relate to their evoked neural activity patterns. We divided healthy volunteers (n= 45) into three groups, two training and one control. Both training groups practiced a set of three different key-press sequences on two days and were subsequently tested on both the trained and a novel set of sequences during fMRI scanning. One

group's training set served as the other group's novel set and vice versa. The control group was tested on the same sequences but did not have any prior practice. Each set contained a pair of similar sequences (based on the same sub-sequences, but in different order) and one different sequence (which shared no sub-sequences with the others) (see section 3.3.2 in Methodological approach for details). During scanning, all sequences were visually cued and paced in an SRT-like task to keep sensory stimulation and net motor output similar across the sequences.

As expected, RTs for trained sequences were faster than those for novel sequences in both training groups. In the control group the corresponding sequences showed no RT difference (Trained vs. Novel, Group 1: t = -9.75, p < 0.001; Group 2: t = -6.55, p < 0.001; Control: t = -1.01, p = 0.329; **Figure 9**). To our surprise, one of the novel sequences (Sequence A) in Group 2 showed a very fast RT improvement during scanning, suggesting that it was learned more easily (**Figure 9, B**).

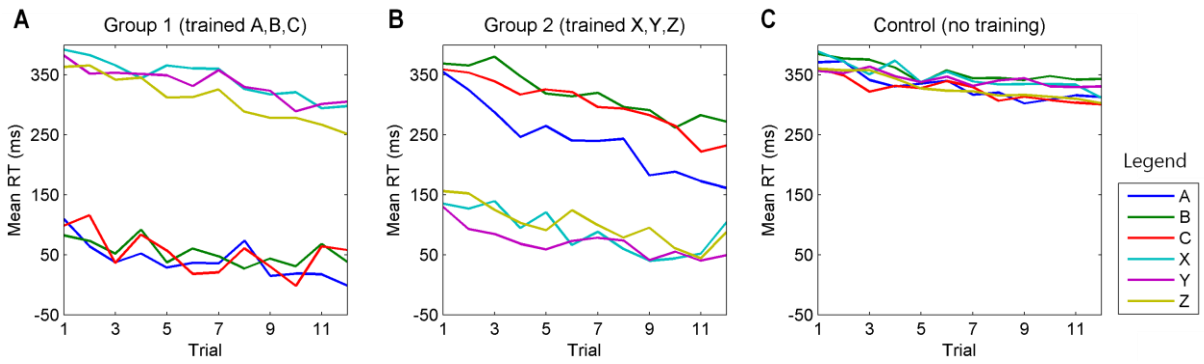


Figure 9. Response times during fMRI scanning. RTs for trained sequences are faster than RTs for novel sequences in both Group 1 **(A)** and Group 2 **(B)**. Note that in Group 2, RTs for sequence A (a novel sequence) decreased more rapidly across trials, suggesting that this sequence was learned more easily. The control group **(C)** shows similar RTs for all sequences.

A univariate fMRI analysis of the pooled data from both training groups revealed increased activity for trained compared to novel sequences in bilateral insula/operculum, precuneus, cuneus, lingual gyrus, left supramarginal gyrus, and right hippocampus (**Figure 10**, red). The opposite contrast, i.e., Novel > Trained sequences resulted in greater activity in bilateral inferior occipital gyrus, left cerebellum, left superior temporal gyrus/angular gyrus, and middle frontal gyrus (**Figure 10**, cyan). In the control group, the same contrasts did not reveal any areas of greater activity (all $p > .05$, *FWE*).

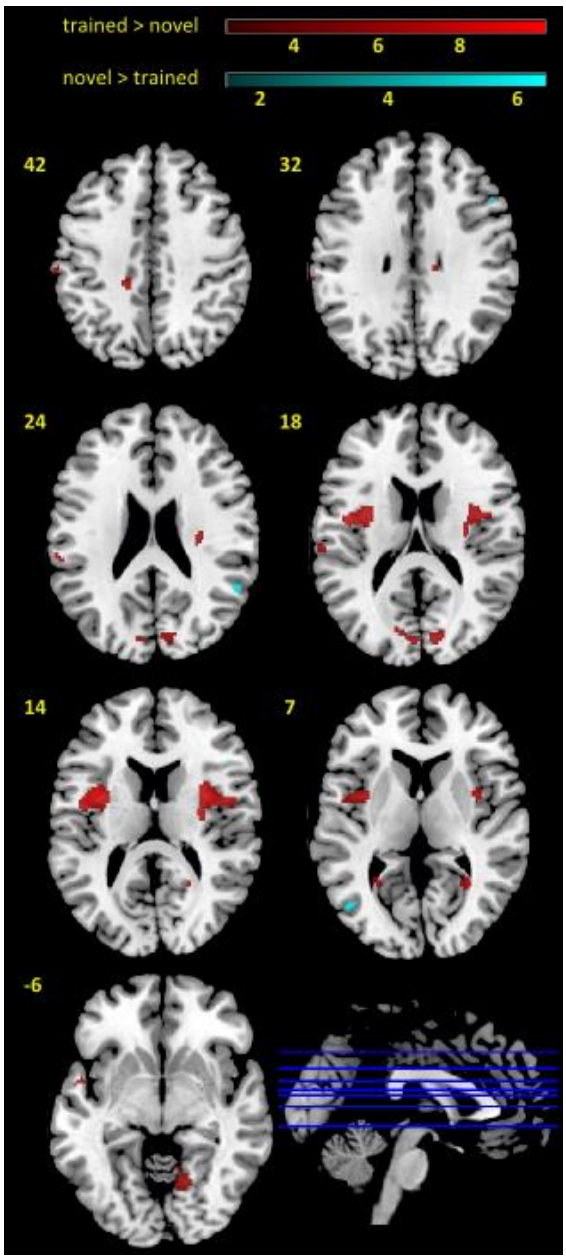


Figure 10. Activity maps for the contrasts Trained > Novel (red) and Novel > Trained (cyan), pooled over both training groups (N= 28) and overlaid onto a single subject anatomical template image. Numbers indicate axial slice number in MNI space. Color bars show t-values. All significance values are corrected for multiple comparisons at the voxel level using Gaussian random field theory as implemented in SPM8.

Since our aim was to go beyond identifying which brain regions are differentially activated by trained and novel sequences, we used RSA to reveal how the representations of individual sequences relate to each other. We performed RSA in three cortical areas (left M1, SMA, and PMC) that are known to be involved in sequential motor performance, as well as in one functionally defined set of voxels that encompassed all voxels that were activated by the task. **Figure 11** shows the resulting correlation matrices for the three anatomical ROIs. In all three brain regions, both training groups show a clear distinction between trained and novel sequences, with higher

correlations within each category than between. The control group does not show any grouping of sequence representations.

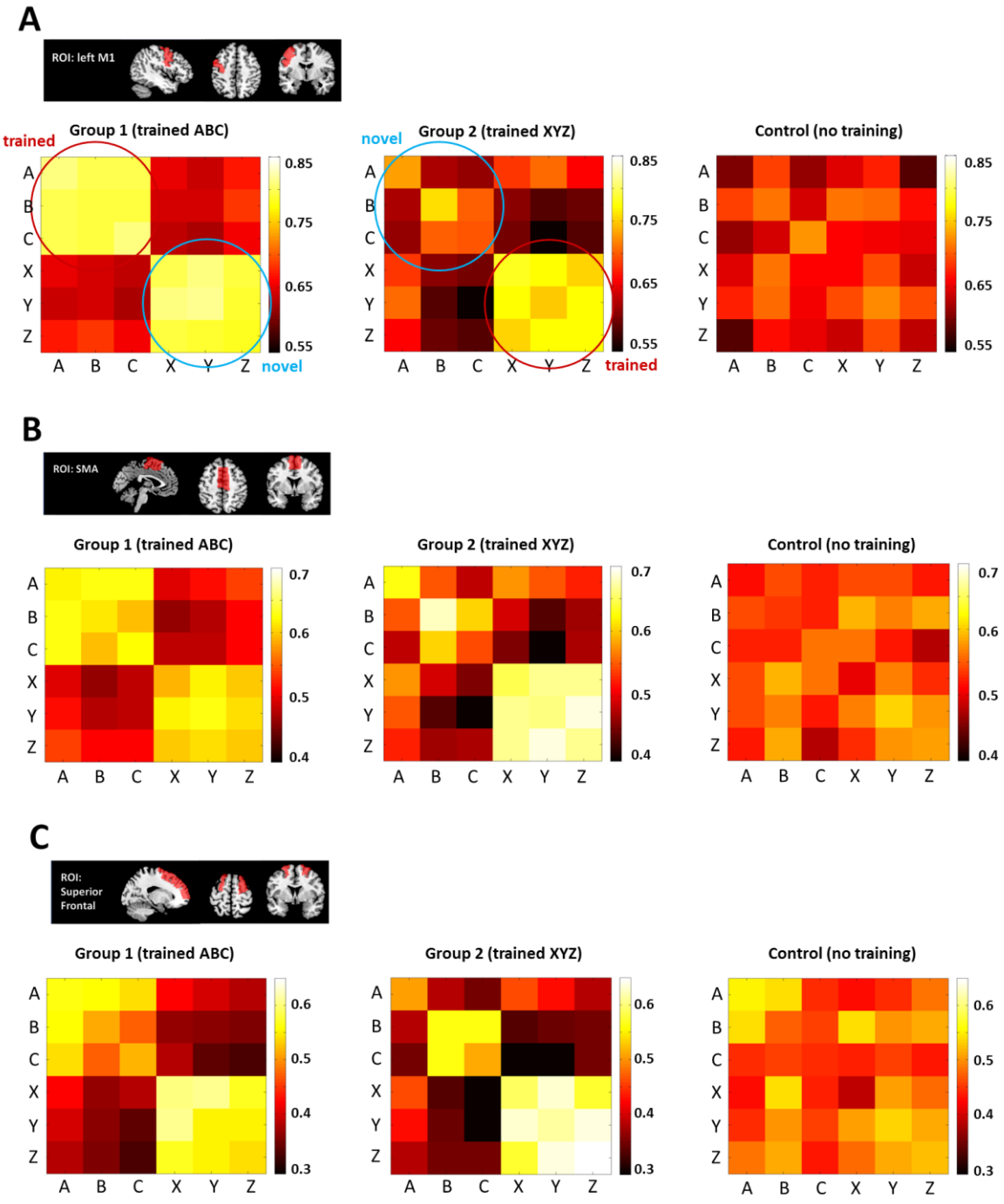


Figure 11. Similarity matrices of fMRI activity patterns. Correlation matrices are shown for Group 1 (left), Group 2 (middle), and the Control group (right). The corresponding ROI is shown above each panel. **(A)** Correlation matrices in M1. Red and blue circles mark correlations among trained and novel sequences respectively. Note the higher within- than between-category correlations in both training groups. The control group shows no such distinction. Note also in Group 2, the unusually low correlation of Sequence A with the other novel sequences and its higher correlation with trained sequences. **(B)** Correlation matrices in SMA. **(C)** Correlation matrices in superiorfrontal cortex.

A somewhat surprising observation was the unusual correlation pattern of Sequence A in Group 2. Note how it correlates relatively less with other novel sequences and more with the trained sequences. Interestingly, this is the same sequence for which RTs decreased more quickly during scanning. When Sequence A was among the trained sequences (Group 1) or presented together with only unfamiliar sequences (Control group) it did not show this unusual pattern. This suggests that the effect was not necessarily due to the sequence structure alone, but that it must be related to how it was perceived or performed in the different groups.

With respect to our question of whether structurally similar sequences are represented by similar activity patterns we did not observe higher correlations between similar sequences than between different ones. Note however, that the correlations between the same sequences (diagonal of the correlation matrix) were also rather low, suggesting that the activity patterns did not contain much information about sequence identity.

To test whether we might have missed any sequence representations by restricting our analysis to the anatomical ROIs, we repeated the same analysis in the entire set of voxels that was activated by the contrast Task > Rest. However, even in these more distributed voxels, RSA showed a similar pattern of sequence representations (**Figure 12**).

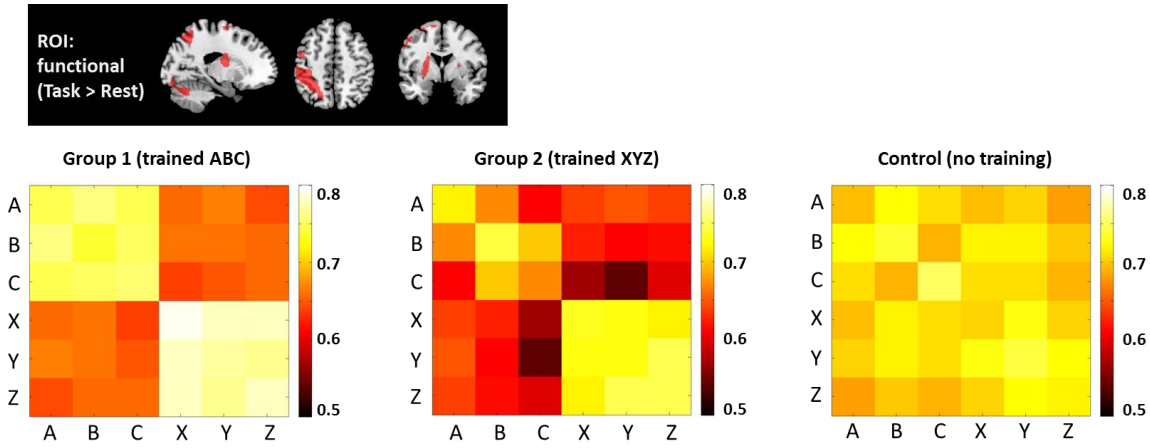


Figure 12. Correlation matrices from the functional ROI based on the Task > Rest contrast. Note that this mask included voxels in a distributed set of brain areas, including sub-cortical structures like the basal ganglia and cerebellum.

Finally, we directly related the neural activity patterns to behavioral differences in sequence performance. For every sequence we calculated the absolute difference between its mean RT in the even split and the mean RT of every sequence in the odd split and vice versa. The resulting dissimilarity matrices (**Figure 13**) reveal a similar pattern as the neural correlation matrices, with small RT differences between sequences of the same category and large differences between categories. Neither RT differences between similar sequences, nor between the same sequences were smaller than those between different sequences of the same category. The unusual

performance on Sequence A in Group 2 is reflected in larger within-category RT differences and smaller between-category differences.

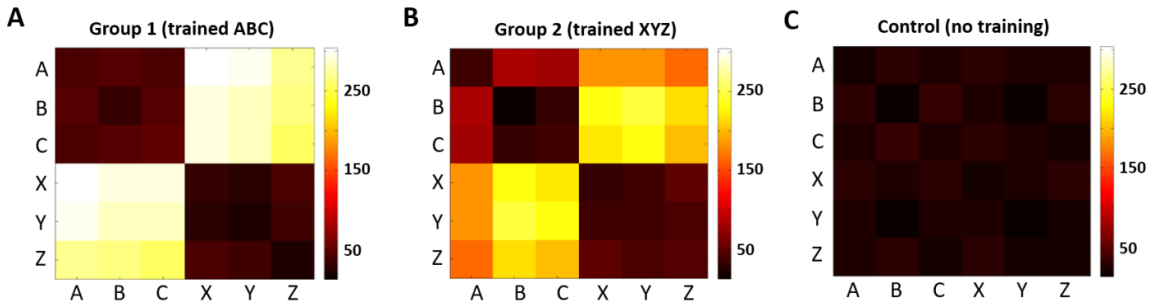


Figure 13. Averaged absolute RT differences within and between sequences across even and odd runs. In both Group 1 **(A)** and Group 2 **(B)** RT differences were largest between trained and novel sequences. In the Control group **(C)** RT differences were small and similar across all sequences.

Spearman rank correlations revealed strong negative correlations between the neural similarity matrices and the RT difference matrices for both training groups and in all ROIs (see **Table 2**). This suggests that the more performance differed between sequences, the more different their neural activity patterns were.

Table 2. Association between RT differences and similarity of neural activity patterns. Rho (and p-values) of the Spearman rank correlation test are shown for each group and ROI.

ROI	Group 1	Group 2	Control
M1	-0.78 (.00005)*	-0.81 (.00001)*	-0.31 (.18)
SMA	-0.73 (.00024)*	-0.78 (.00004)*	-0.14 (.54)
Superior Frontal	-0.75 (.00014)*	-0.8 (.00002)*	-0.25 (.26)
Task-Rest	-0.81 (.00001)*	-0.79 (.00003)*	-0.38 (.09)

*significant at $p < .001$

4.3 Study III

The aim of **Study III** was to dissociate the preSMA's role in sequence chunking from its role in hand switching. In both human (Hikosaka et al. 1996a; Kennerley et al. 2004; Sakai et al. 1999) and non-human primates (Nakamura et al. 1998) the preSMA is involved in motor sequencing and in the initiation of motor chunks. However, since the preSMA has also been associated with a number of other functions such as movement inhibition (Chen et al. 2009; Obeso et al. 2013; Picazio et al. 2014), task switching (Roberts and Husain 2015; Rushworth et al. 2002), and movement initiation (Mita et al. 2009), its role in motor chunking might reflect some common underlying processes, rather than chunking per se. Previous TMS studies that investigated the role of the preSMA in

motor chunking did not differentiate systematically between chunking and hand-switching (Kennerley et al. 2004) or between chunking and sequence initiation (Ruitenberg et al. 2014b). In **Study III** we designed a sequencing task that allowed us to dissociate motor chunking from hand switching by testing all possible combinations of chunk boundaries (between or within chunks) and switch types (no switch, towards, or away from target hand). Moreover, we analyzed sequence initiation elements separately from chunk initiation points.

We reasoned that if the left preSMA is necessary for sequence chunking, a temporary disruption via dTMS would interfere with processing of the next chunk, (i.e., delay the next response irrespective of hand-switch condition), but only if stimulation was delivered between chunks. Alternatively, if the left preSMA's role is more related to hand switching, we would expect TMS to delay responses in all conditions involving a hand-switch, independent of chunk boundary. In contrast to the two possible outcome scenarios for preSMA stimulation, we expected TMS over left M1 to delay all responses with the right hand, i.e., to interfere with contralateral finger movements irrespective of whether they involve a hand-switch or a chunk boundary.

The average RTs for each sequence element revealed that participants chunked the sequence in the intended format, as can be seen by the increased RTs for the 1st, 5th, 9th, and 13th element in **Figure 14**. We found that dTMS before the first key-press (120 ms after the onset of the visual sequence cue) decreased RTs for sequence initiation at both stimulation sites. There was a significant effect of the factor Stimulation Condition in an rmANOVA on the first sequence element [$F(1.7,28.4) = 14.78$, $p < 0.001$]. Planned pairwise comparisons between each stimulation site and the no TMS condition were also significant [M1 vs. no TMS: $t(17) = 3.9$, $p = 0.002$; preSMA vs. no TMS: $t(17) = 6.8$, $p < 0.001$, Bonferroni corrected].

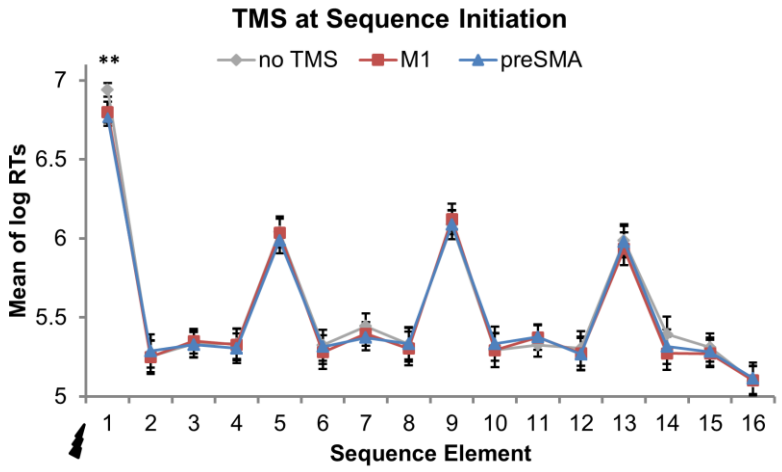


Figure 14: TMS at sequence initiation. RTs for the first sequence element are decreased after TMS stimulation (flash symbol) over both left M1 and left preSMA. Note also the long RTs for the 5th, 9th, and 13th elements, which correspond to the start of each chunk. rmANOVA for the difference between stimulation sites was performed on the first sequence element. Error bars show SE. ** M1 vs. no TMS and preSMA vs. no TMS were both significant at $p < 0.05$, Bonferroni corrected.

In the *Between chunks* condition, we found that dTMS over preSMA delayed the next response only in the *Switch Towards* condition and not in the *Stay on Right* or *Switch Away* conditions (**Figure 15, A**). In contrast, stimulation over M1 slowed responses in all three switch conditions (*Switch Towards*: rmANOVA: $F(1.5,25.9) = 5.65$, $p < 0.014$; post-hoc pairwise comparisons: M1 vs. no TMS: $t(17) = 4.0$, $p = 0.002$; preSMA vs. no TMS: $t(17) = 2.8$, $p < 0.022$; *Stay on Right*: rmANOVA: $F(1.6,27.5) = 8.6$, $p < 0.02$; post-hoc pairwise comparisons: M1 vs. no TMS: $t(17) = 3.7$, $p = 0.004$; preSMA vs. no TMS: $t(17) = 0.7$, $p < 0.89$; *Switch Away*: rmANOVA: $F(1.8,30.4) = 3.4$, $p = 0.051$; post-hoc pairwise comparisons: M1 vs. no TMS: $t(17) = 2.5$, $p = 0.042$; preSMA vs. no TMS: $t(17) = 0.9$, $p = 0.72$; all pairwise comparisons are Bonferroni corrected for the number of planned comparisons).

In the *Within chunks* condition dTMS over preSMA did not lead to any significant RT changes in any of the switch conditions (**Figure 15, B**). Stimulation over M1 delayed the next response in the *Switch Towards* and *Stay on Right* conditions, but not in the *Switch Away* condition (*Switch Towards:* rmANOVA: $F(1.4,23.2) = 9.73$, $p < 0.002$; post-hoc pairwise comparisons: M1 vs. no TMS: $t(17) = 3.3$, $p = 0.008$; preSMA vs. no TMS: $t(17) = 0.5$, $p = 1$; *Stay on Right*: rmANOVA: $F(1.3,21.5) = 18.5$, $p < 0.001$; post-hoc pairwise comparisons: M1 vs. no TMS: $t(17) = 4.4$, $p = 0.001$; preSMA vs. no TMS: $t(17) = 1.0$, $p = 0.66$; *Switch Away*: rmANOVA: $F(1.4,23.8) = 3.4$, $p = 0.065$; all pairwise comparisons are Bonferroni corrected for the number of planned comparisons). Although not part of our initial hypotheses, we observed a delay in the onset of the next chunk (i.e., in the third response after TMS) in the *Stay on Right* and the *Switch Away* conditions after M1 stimulation (**Figure 15,** middle and bottom row). An rmANOVA on the third element after the TMS pulse confirmed that this delay was significant (*Stay on Right:* $F(1.9,31.5) = 5.5$, $p = 0.01$; pairwise comparisons: M1 vs no TMS: $t(17) = 2.7$, $p = 0.03$, preSMA vs. no TMS: $t(17) = 0.4$, $p = 1$; *Switch Away from Right*: $F(1.6,27.8) = 7.4$, $p = 0.004$; pairwise comparisons: M1 vs no TMS: $t(17) = 3.4$, $p = 0.006$, preSMA vs. no TMS: $t(17) = 0.4$, $p = 1$).

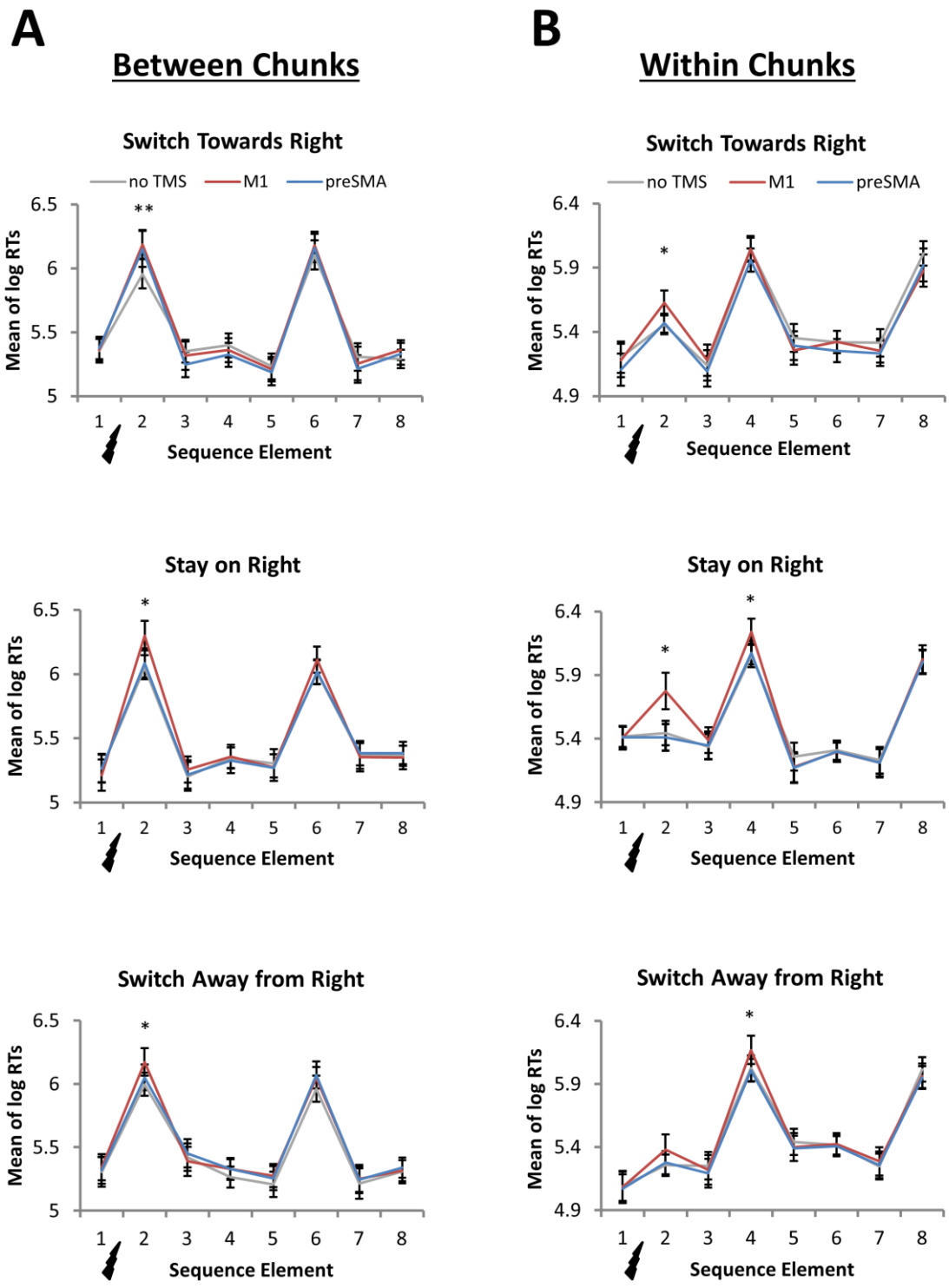


Figure 15. Effect of TMS Between and Within Chunks. All trials were aligned to the element preceding the TMS pulse (flash symbol). rmANOVAs were performed on the sequence element after the TMS pulse, i.e., element 2. **(A)** In the *Between-chunks* condition, TMS over both M1 and preSMA delayed RTs when applied before a switch to the right (A, top). When no switch (A, middle) or a switch away from the right hand (A, bottom) was required, only TMS over M1, but not over preSMA, increased RTs. **(B)** In the *Within-chunks* condition, TMS over M1 led to increased RTs when a switch towards the right hand (B, top) or a stay on the right hand (B, middle) was required, but not when applied before a switch away from the right hand (B, bottom). Stimulation over preSMA did not delay RTs in any of the switch conditions. Note also the significant RT increases for

the onset of the following chunk, i.e., 3rd element after TMS, for M1 stimulation in the Stay on Right (B, middle) and Switch Away from Right (B, bottom) conditions. Error bars show SE. *M1 vs. no TMS is significant at $p < 0.05$, ** M1 vs. no TMS and preSMA vs. no TMS are both significant at $p < 0.05$, all values are Bonferroni corrected.

Because we found that preSMA stimulation delayed responses when applied between chunks but not within chunks in the *Switch Towards* condition, we inspected this effect more closely. An rmANOVA with factors Stimulation Site (no TMS, M1, preSMA), Chunk Position (Between, Within), and the interaction term showed a trend for an interaction [$F(1.92,32.64) = 3.16$, $p = 0.058$], suggesting that preSMA stimulation only delays RTs between chunks, but not within them (see **Figure 16**).

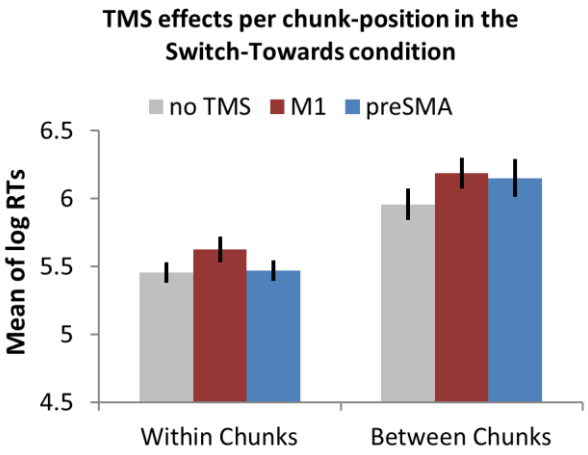


Figure 16: Interaction between TMS site and Chunking Position. The direct comparison of Within-chunk TMS and Between-chunk TMS for the three stimulation sites shows a strong trend towards a significant interaction ($p = 0.058$) between TMS-site (no TMS, M1, preSMA) and Chunk-position (Within chunks, Between chunks) when switching towards the right hand. Note that TMS over M1 delayed RTs at both chunk-positions, whereas TMS over preSMA only delayed RTs when applied between chunks. Error bars show SE.

5 DISCUSSION

5.1 Brief summary and discussion of Study I

In **Study I** we found that variable sequence practice leads to more task-general transfer than constant practice. Moreover, after variable, but not constant, practice the amount of skill transfer correlated with how much participants improved during practice. Regarding the context dependency of sequence specific knowledge, we found greater skill transfer to an overlapping sequence and specifically to the predictable (last) element of shared triplets.

Why would variable practice be more beneficial for task-general transfer? After all, both groups were exposed to the same total amount of practice. To interpret this result it may be helpful not to ask why variable practice is better, but rather why constant practice is worse. Constant practice led to the acquisition of more sequence-specific knowledge, which in turn might have caused more negative transfer, i.e., expectations from the trained sequence interfering with performance on a novel sequence. Although generally not a widely studied topic in sequence learning, negative transfer effects have been reported when participants switched from sequential to random SRT trials (Robertson 2007) and for sequential rule application tasks (Woltz et al. 2000). Our observation that training gains correlated with transfer only in the Variable group further supports the idea that transfer in the Constant group suffered from interference effects. Thus, it seems plausible that both groups developed similar task-general skills, but that performance in the Constant group was delayed due to (implicit) expectations of a different stimulus order.

A common explanation for the benefit of variable practice schedules is that they require motor plans to be actively reconstructed with every switch between tasks and that these higher demands on working memory lead to better skill encoding (Cross et al. 2007; Immink and Wright 1998; Lee and Magill 1983). Here, we observed a similar advantage of variable practice, but for an implicitly learned skill. This is difficult to reconcile with an effortful motor plan reconstruction in working memory. A possible explanation for this might be that the observed contextual interference effect rests on different mechanisms, depending on whether a skill is learned explicitly or implicitly. This hypothesis, i.e., that for explicitly learned skills, variable practice is advantageous because it leads to better skill encoding, whereas for implicit skills the advantage is due to less interference, could be tested by directly comparing the transfer patterns for explicitly and implicitly learned sequences. Compared to implicitly learned sequences, explicit learning should abolish the interference (i.e., lead to more task-general transfer) in the Constant group (see **Figure 17** for a graph of the expected outcome).

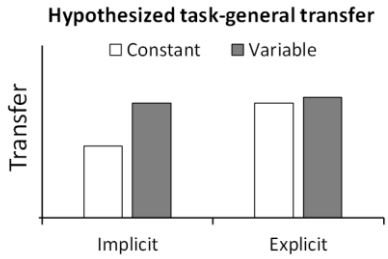


Figure 17. Hypothesized outcomes for task-general transfer after implicit and explicit sequence practice. For implicit tasks, constant practice reduces transfer due to interference from unconscious sequence expectations. However, such an effect would not be expected after explicit practice, where sequence expectations can be adjusted consciously.

The finding that sequencing skills for shorter sub-sequences can transfer to a novel sequence context is consistent with commonly observed part-whole transfer, where certain elements of a task are first practiced in isolation and are then integrated into the full task (Schmidt and Lee 2005; Spruit et al. 2014). Moreover, Jiménez et al. (2006) previously demonstrated sequence-specific transfer when an implicitly practiced sequence is embedded into random stimuli. Here, we extended these findings by showing similar transfer effects also for sequence fragments. The amount of sub-sequence overlap is thus an important point to take into consideration when designing control and transfer sequences.

5.1.1 Limitations

One limitation of this study is that we could not exclude the possibility that participants became aware of the sequential structure of the stimuli. Our task was designed to avoid the development of explicit sequence knowledge as much as possible, but we cannot be certain that the described effects relate to purely implicit knowledge. However, it seems reasonable to assume that implicit skill accounted for a large part of the performance improvements, especially considering the length and complexity of the sequences. Importantly, previous studies did not find any relation between the amount of transfer and differences in explicit sequence knowledge (Sanchez et al. 2015; Song et al. 2012).

Further, one might argue that the amount of motor learning might be better captured in a delayed retention test (e.g., after 24 h), than at the end of training. As pointed out in the introduction of this thesis, performance at the end of a practice session may be more strongly affected by transient factors that are not representative of the actual amount of learning (Kantak and Winstein 2012). It would thus be interesting to evaluate the persistency of the observed transfer effects, by using a delayed retention test (see section 5.4 on Future Directions).

5.2 Brief summary and discussion of Study II

In **Study II** we found that trained and novel motor sequences were represented by distinct patterns of activity, even in brain areas that showed no such discrimination in their mean level of activity. Further, we found that the activity patterns of individual sequences reflected similarities in performance speed, but not structural similarities in sequence content.

Our results confirm earlier reports about distinct representations of trained and novel sequences (Huang et al. 2013; Wiestler and Diedrichsen 2013). Additionally, we were able to show that this difference was not due to differences in response rate, because all sequence elements were cued at a fixed inter-stimulus interval. Moreover, this distinction could not be explained by differences in inherent sequence properties such as difficulty, because the sequences were represented similarly when they were all unfamiliar (i.e., in a control group without previous sequence training).

We found no indication that structurally similar sequences were represented by similar activity patterns. This was somewhat surprising, given that one could have expected that sequences that consist of the same sub-sequences also recruit similar neural circuits. While one explanation might be that sub-sequences recruit different neural circuits depending on their sequence context, it is important to point out that different trials of the same sequence did not evoke very similar representations, either. A possible reason for the low consistency of individual sequence representations might be that two days of sequence training were not sufficient to form stable neural circuits for each sequence. Alternatively, the task format (i.e., an SRT-like task with a slow ISI) might have promoted a different kind of sequence retrieval than the DSP-like tasks used in previous studies on sequence representations (Huang et al. 2013; Wiestler and Diedrichsen 2013; Wymbs and Grafton 2015). Fast sequence execution in DSP-like tasks is likely to require a full sequence representation in working memory, whereas sequence retrieval in SRT-like tasks is based more strongly on serial associations between individual elements (Abrahamse et al. 2010; Verwey and Abrahamse 2012). Such associative and temporally dynamic sequence retrieval may explain why sequence representations were less stable in our study.

While neural activity patterns did not reflect structural sequence similarities, they showed a clear association with behavioral performance in both training groups. This finding was further supported by the unexpected finding of a quick RT decrease for Sequence A in the novel condition. Corresponding to this irregular behavioral performance, we observed that its neural activity pattern was also different, showing relatively high correlations with trained sequences and lower correlations with novel sequences. Thus, our findings suggest that the neural activity patterns of individual sequences reflected behaviorally relevant information, rather than sequence content.

5.2.1 Limitations

Another limitation, in addition to the previously mentioned concerns about training duration and task paradigm, is that participants' chunking patterns were not well controlled. Although we

encouraged participants during training to chunk sequences in a way that would result in the same chunks for the similar sequences, it is not clear whether participants adhered to this structure during testing. Indeed, Jiménez et al. (2011) showed that chunking patterns can disappear when an initially chunked sequence is performed in an SRT task without external grouping cues. A different chunking format could have reduced the (perceived) similarities between the sequences, thereby also affecting their neural representations. Further, it seems likely that information about sequence content is also encoded in the temporal modulation of the neural response. However, since a voxel's variability over time (within a trial) is not captured by its regression parameter, we could not capture such information.

5.3 Brief summary and discussion of Study III

In **Study III** we found that TMS stimulation over left preSMA delayed the next response, but only if the stimulation was applied between chunks, and if the following response required a hand-switch toward the contralateral (right) hand. This specific effect for the condition where both an initiation of a new chunk and an initiation of a movement with the other hand (switch towards) were required was in contrast with the effects we observed after M1 stimulation. TMS over left M1 slowed down all responses with the contralateral hand, irrespective of chunk boundary or hand-switch requirements. As opposed to the disruptive effects of TMS during sequence performance, stimulation before sequence initiation improved RTs at both stimulation sites.

These findings suggest that proper functioning of the left preSMA is crucial in complex tasks that require both chunk initiation and a switch between hands. Neither chunking nor hand switching alone seemed to be demanding enough to be affected by preSMA stimulation. Our results are in line with previous reports of a context dependent influence of preSMA on M1 (Mars et al. 2009; Neubert et al. 2010). In these studies, a paired-pulse paradigm was used to reveal a facilitating influence of preSMA on M1 (i.e., preSMA stimulation increased MEP amplitude for the correct response hand), but only when participants had to inhibit a prepared response with one hand and switch to the other hand instead. Interestingly, neurons in the preSMA of nonhuman primates have been found to have firing properties that would enable them to inhibit a motor response, but not to initiate a movement (Scangos and Stuphorn 2010). Thus, in connection with previous research our results suggest that the preSMA's role in motor sequence performance is not necessarily to initiate new motor chunks, but rather to help select appropriate responses, especially in the context of dynamic task requirements.

Finally, our finding that TMS over preSMA decreased RTs for the first sequence element contrasts with previous reports in which preSMA stimulation increased RTs for sequence initiation (Kennerley et al. 2004; Ruitenberg et al. 2014b). A possible explanation for this discrepancy might lie in the timing of the stimulation. Given the relatively long and complex motor sequence in our study, stimulation at 120 and 160 ms post cue onset might have been too early to interfere with

sequence preparations. Instead, responses could have been facilitated due to altered cortical excitability thresholds. A similar time-dependency of the direction of the TMS effect has been reported for simpler choice-reaction time tasks, where early TMS (i.e., soon after the 'go' cue) decreased RTs, while late stimulation increased RTs (Leocani et al. 2000; Pascual-Leone et al. 1992; Ziemann et al. 1997). Alternatively, the improved RTs might reflect intersensory facilitation effects due to the clicking sound of the TMS or somatosensory stimulation over the scalp. Additional sensory stimulation has been shown to decrease RTs (Nickerson 1973; Terao et al. 1997).

5.3.1 Limitations

One limitation in this study was the relatively mild stimulation (double-pulse) that was used to interrupt preSMA processing. It is possible that a more powerful inhibition paradigm, such as theta-burst stimulation, would have revealed preSMA involvement in other conditions as well (e.g., for chunking or hand-switching alone). Nevertheless, we can conclude that the joint requirement of chunking and hand-switching was most susceptible to preSMA stimulation.

Another limitation relates to the spatial specificity of the TMS effect. Although the stimulation site was defined for each participant individually (based on their anatomical MRI), we cannot be certain that we always stimulated the left preSMA in each participant. Since the coil was always positioned just anterior of the SMA-preSMA boundary, it is possible that in some participants the stimulated area corresponded more closely to SMA than to preSMA. Further, the close proximity to the midline makes it possible that the right hemisphere received some stimulation as well. However, the coil orientation and the brief duration of the stimulation suggest that the main effect of the stimulation was focused on the left preSMA.

Finally, since the different types of chunk boundaries had to be strictly controlled and counterbalanced, we could not let participants develop spontaneous chunking patterns. Therefore, one might argue that the imposed chunking structure in our task required the concatenation of sub-sequences, rather than chunking in the sense of segmenting a longer sequence into shorter parts. However, it seems reasonable to assume that many of the underlying processes, such as selecting a new chunk, preparing its individual elements, and suppressing competing motor plans, are similar for externally imposed and self-induced chunk boundaries.

5.4 Future Directions

While the work in this thesis was an attempt to provide more insights into the mechanisms of sequence learning, it seems to have generated more questions than answers. In the following, I will briefly mention some of the questions that would need to be addressed in future studies.

In **Study I** we evaluated implicit transfer immediately after sequence practice. Therefore, it was not possible to determine whether the observed interference after constant practice was a transient effect or whether performance at later time points would have been equally affected. Constant practice could have reduced task-general transfer due to task set effects (i.e., due to a set of cognitive processes that is maintained throughout a task and that reflects the overall task goal and expectations about specific stimulus and response features) (Monsell 2003; Sakai 2008). Such task set effects, as well as switch costs (i.e., increased RT and error proneness right after switching to a new task), would be expected to interfere with transfer performance only immediately after practice. Transfer at retention, e.g., 24 h after practice, would then be expected to be similar after constant and variable practice. Alternatively, if constant practice has a more permanent effect on task-general transfer, its disadvantage should still be present after a longer retention period. Thus, although retention and transfer tasks are often mentioned as different alternatives to measuring either the temporal stability or the generalizability of a learned skill (Kantak and Winstein 2012; Schmidt 2005), their combination i.e., testing transfer at multiple time-points, could provide further insights into the factors that promote or impede skill transfer.

Further, it would be interesting to explore how modality-specific possible interference effects are. Since sequence knowledge can be represented in different modalities, i.e., stimulus-based, response-based, and based on stimulus-response couplings, it would be interesting to evaluate how these components are affected by interference from performing a subsequent task in only one domain (e.g., only attending to visuo-spatial stimuli).

Another question that would need further investigation concerns the role of chunking in skill transfer. **Study I** demonstrated skill transfer when sub-sequences of familiar sequences are inserted into a novel sequence context. However, for chunked sequences, it remains unclear whether the chunk content or the chunk size (i.e., the number of elements within a chunk) is more dominant when it comes to transfer. For example, assume that a sequence is practiced as 231 – 41 – 342 (i.e., with chunk sizes of 3, 2, and 3 elements). How would a transfer sequence, in which the order of the last two chunks is reversed, be chunked? Would the chunk format be preserved, at the cost of breaking up chunk content, i.e., resulting in a 231 – 34 – 241 pattern? Or would chunk content be preserved, resulting in a new chunk format, i.e., 231 – 342 – 41? A better understanding of the interactions between chunk format and chunk content would have practical implications for the design of training paradigms and could also be relevant for computational models of how sequencing skills are learned.

In **Study II** we used a sequencing task with a relatively long (900 ms) ISI, to allow participants enough time for a correct response and to ensure that the total time spent on task was similar for trained and novel sequences. Except for the fact that the training sequences were practiced explicitly, our task was relatively similar to an SRT task. Other studies that investigated the neural representation of motor sequences have used more DSP-like tasks with shorter sequences and faster performances, which might have prompted different retrieval modes (see Discussion for Study II). It

is often difficult to interpret and compare results between studies that used slightly different tasks and training paradigms. Therefore, it seems that the sequence learning community would greatly benefit from a direct comparison of the neural representations underlying sequence performance in both SRT- and DSP-like tasks.

We did not observe a relation between structural sequence similarity and neural activity patterns in **Study II**. However, since it is not clear whether possible similarities were precluded by the task paradigm (i.e., slow responses that did not encourage chunking), it would be interesting to investigate whether sequence similarities would be reflected in neural activity if the structural similarity was more pronounced. For example, two sequences consisting of only two chunks, but in reversed order (e.g., AB and BA) might be represented more similarly if a speedy and chunked performance is encouraged.

In **Study III** we found that preSMA stimulation delays the initiation of the next chunk when a simultaneous hand-switch was required. In this study, TMS pulses were always triggered by the response to the preceding sequence element. Thus, the timing between the TMS pulse and the onset of the (planned) response varied between trials. This made it difficult to draw conclusions about the time frame during which the preSMA is involved in chunking. More specifically, it would be interesting to investigate whether the preSMA is already involved in motor planning and action selection while a current chunk is still being executed (i.e., in parallel to motor performance), or whether it is recruited only during the pause between adjacent chunks (i.e., serial recruitment). Behaviorally, it has been shown that the duration of the pause between chunks decreases with further training (e.g. Wymbs et al. 2012), which allows for faster and smoother sequence performance. This could be achieved in two ways, either chunks are concatenated into increasingly longer units that eventually span the entire length of the sequence (i.e., chunk boundaries will eventually dissolve completely), or chunks are already prepared and loaded while another movement is still ongoing, allowing for minimal to no delay between consecutive chunks (i.e., performance appears seamless, but sequence information is still retrieved in chunks). These two alternatives would lead to different predictions regarding the preSMA's involvement in later learning stages. If chunk boundaries eventually dissolve, preSMA stimulation should also lose its disruptive effect on RTs. Alternatively, if sequence knowledge is still retrieved in chunks, then preSMA stimulation should still be effective, but at earlier time-points.

5.5 General discussion and conclusions

This thesis investigated several behavioral and neural aspects of how we learn and perform sequences of movements. As learning always takes place within a given context, it is important to understand how this context shapes what we learn. The physical surrounding, task instructions, expectations, the training schedule and many more factors may be linked to the ultimate training outcome. In experimental set-ups, learning and performance are often measured under very similar

conditions, whereas many real-life tasks require a more flexible application of skills. One way to increase (general) skill transfer would thus be to alternate practice between different tasks (**Study I**).

Further, factors that affect behavioral performance also affect the neural representation of a skill (**Study II**). This was demonstrated by the fact that motor sequences that were performed at similar speeds were also represented by more similar patterns of neural activity. In some theories about neural mechanisms of motor sequence learning the importance of contextual factors has been somewhat neglected. For example, Wiestler and Diedrichsen (2013) suggested that sequence learning leads to the formation of specialized neural circuits that represent the transition between two or three fingers, rather than just an individual finger movement. Therefore, sequences that require different transitions would activate different neural circuits, allowing for distinct sequence representations. However, such a model would fail to explain how sequences that consist of the same transitions could have distinct representations or how a musician can learn to play many different songs. To allow for learning of more than one sequence, the formation of specialized neural circuits would have to be dynamic and in close association with the context of the learned skill, such as physical cues, goals, etc.

Finally, chunking is not only an important behavioral strategy in motor sequencing, it can also reveal some of the mechanisms of skilled performance. Chunking per-se did not seem to be critically dependent on preSMA functioning, but with the added task complexity from hand-switching, preSMA involvement became necessary (**Study III**). Thus, even relatively small changes in task complexity can influence how chunking is implemented at the neural level. Again, this emphasizes that the representation of a skill is dynamically modulated by the context within which the skill is performed.

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